THE EXTENT AND IMPACT OF AVIAN POX ON SILVEREYES (*ZOSTEROPS LATERALIS*) IN NEW ZEALAND

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General Abstract

The main objective of my investigation was to ascertain the prevalence and impact of avian pox in New Zealand silvereyes (*Zosterops lateralis*). Silvereyes were mist-netted between 2010 and 2012 in three locations of the South Island and were examined for external signs of avian pox. Of the 409 silvereyes captured over this time period, avian pox lesions were identified in 9 birds (2.2%) and were found in both sexes as well as in juveniles. The highest incidence of avian pox was seen on the west coast at Moana (7.2%) between late May and mid-June 2011. Overall prevalence for each location over the period of the survey ranged from 1% in Kaikoura, Christchurch 3.6% and Moana 3.5% suggesting only slight differences between populations. I next investigated the prevalence of avian pox in a community of forest passerines at Kowhai Bush Kaikoura in the spring of 2012. Pox lesions were observed in 2/492 of the birds caught. Both cases (3.8%, n=52) were in the introduced chaffinch (*Fringilla coelebs*). Pox occurred in < 1% of individuals of the introduced species (n=313) and was absent in all native and endemic species (n=189). No pox lesions were seen in silvereyes captured in the 2012 Kaikoura survey (0/69). White blood cell profiles, fat scores and mass where also compared between silvereyes with and without avian pox lesions but no differences were found, however, the small sample of individuals with pox meant the power to detect a difference was low. A review of the literature on avian pox revealed a similar low prevalence of avian pox to that seen in this study and the prevalence of avian pox seen in other populations of white-eyes in New Zealand and elsewhere. However, across a range of other passerine species, avian pox was significantly more prevalent in geographically isolated endemic species than other groups. My investigation suggests that avian pox needs to be monitored over extended periods to estimate its prevalence and how its epizootiology is influenced by a variety of biotic and abiotic factors. Furthermore, my investigation reveals the need for more information, including the identification of the type and virulence of strains present in New Zealand and the role that silvereyes and introduced birds may have on the dynamics of avian pox in rare endemic birds.

Introduction

Infectious Avian Diseases

A profound number of infectious viral, bacterial and fungal diseases impact on the well being of domestic and wild avian species. Infectious diseases are dynamic entities that challenge our ability to deal with both the diseases we already know about as well as emerging diseases. Interactions between humans, domestic animals and wildlife play an integral part in how diseases continue to develop, spread and ultimately affect the health and survival of these groups (Daszack et al. 2000). Understanding the etiology and epizootiology of diseases present in wildlife, including birds, is important because wild animals can act as a reservoir for pathogens that affect human health, domestic animals and other wild species (Daszack et al. 2000). Significant gaps exist in our understanding about the impact of infectious diseases in wild birds and the role they play in compromising the survival of many of the world's endangered avifauna.

Avian Pox

Avian pox is a viral disease afflicting a wide range of bird species throughout the world. A review of the literature on the worldwide prevalence of this disease (van Riper & Forrester 2007) reveals that it has been detected in as many as 278 species covering 20 different orders. The pox virus found in birds belongs to the genus *Avipoxvirus* belonging to the Subfamily Chordopoxvirinae of the Family Poxviridae (Tripathy 1993) It is regarded as one of the largest viruses and is approximately 380 X 280 X

200 nm in size (Tripathy & Reed 2008). Recent advances in molecular techniques have led to the identification of distinct strains of the virus and currently, 10 strains of avian pox have been listed by the International Committee on Taxonomy of Viruses (ICTV) (Weli & Tryland 2011). The type species for the genus *Avipoxvirus* is Fowlpox (FPV) Tripathy & Reed 2008) and some of those listed by ICTV include, canary, mynah, pigeon, quail, sparrow, starling and turkey pox (Weli &Tryland 2011). Empirical evidence suggests that to a large degree many strains are host specific (Tripathy & Reed 2008).

Avian pox is highly infectious, resistant to extreme conditions and can survive in dry scabs for very long periods, from months to years (Tripathy 1993). Transmission of the disease from bird to bird is most likely to occur through direct contact with the mouthparts of biting arthropods. Mosquitoes are well known vectors of avian pox (van Riper et al. 2002). Abrasions on the skin can also allow pox viruses to penetrate the epidermis and it is possible for the disease to be passed on via contaminated perches (Bleitz 1958) or via aerosol particles in a confined environment, such as an aviary (Mete et al. 2001).

Two main forms of avian pox are known to exist and the most devastating of these is the diphtheritic or wet variant (van Riper & Forrester 2007). Infection with diphtheritic pox in domestic chickens and other species has been reported to lead to the growth of lesions on mucous membranes (Bolte et al.1999). The lesions rapidly increase in size and secondary

bacterial infection can then spread to the upper respiratory tract (van Riper & Forrester 2007). The cutaneous or dry form of avian pox manifests itself in the form of pock-like lesions that grow on the unfeathered parts of the skin (Bolte et al.1999). These wart-like growths are caused by prolific generation of epithelial cells and as they 'stack' this leads to the formation of a lesion (van Riper & Forrester 2007). Lesions may burst with subsequent bacterial infection and necrosis. Similar secondary infections in some cases can lead to the loss of one or more digits (van Riper et al 2002; pers. obs.). Although many birds recover from the disease younger birds are considered to be the most vulnerable (e.g. Vargus 1987). Lesions that grow around the eyes can impair vision and compromise a bird's ability to forage (e.g. Karstad 1965). Typically one or two lesions are seen at a time in wild birds and it has been suggested that this is an indication of a long established host-parasite relationship where the virus benefits from being carried by an individual for an extended period (van Riper & Forester 2007).

Identifying Avian Pox

Advances in molecular techniques can now allow identification of avian pox using polymerase chain reaction (PCR) testing (e.g. Luschow et al. 2004). In the past a common technique was to grow the virus on the chorioallantoic membranes of chicken embryos (e.g. Docherty et al. 1991). In infected individuals, intracytoplasmic inclusions (also known as 'Bollinger bodies') may be seen using electron microscopy (e.g. Kirmes 1966), however, these inclusions are not always seen and so the

technique is not always reliable (van Riper & Forrester 2007). The inclusions are caused by the migration of virions to vacuoles where they become coated with an outer membrane (van Riper & Forrester 2007). At the simplest level, lesions can be identified visually although it has been suggested that lesions caused by bacteria or mites are sometimes very similar (van Riper & Forrester 2007). Nevertheless, visual inspection has the advantage in that it can be used to rapidly assess the presence of pox in a population.

Consequences of Avian Pox in Wild Birds

While the epizootiology, prevalence and impact of FPV has been well studied in domestic poultry, little is known about how these findings apply to wild birds where a wide variety of strains are in circulation (van Riper & Forester 2007). Protection against the disease in chickens and turkeys is important as virulent forms of the disease can infect an entire flock (Tripathy & Reed 2008). Apart from the probable high mortality rate if the diphtheritic form is present in a flock, considerable losses can also be expected if the milder cutaneous form persists in a flock, particularly if secondary infection or lesions grow around the eyes (Tripathy & Reed 2008).

In contrast to domestic birds our understanding of the epizootiology and effects of the various strains of avian pox in wild bird populations is limited. However, there is no shortage of literature discussing the presence of pox in a variety of wild species of birds. A number of recent

studies have even investigated the role of disease as a cause of extinction of some avian species and have highlighted the role played by epizootics, such as avian pox (e.g. Jenkins et al. 1989; van Riper et al. 2002, Smits et al. 2005, Kleindorfer & Dudaniec 2006). A common factor in these investigations is that the populations concerned are all endemic species and inhabit geographically isolated islands. For example, in Hawaii there is strong evidence to suggest that the introduction of exotic birds carrying avian pox in the 1800s and the introduction of the ornithophilic mosquito *Culex quinquefasciatus* (an effective vector) led to rapid declines of endemic species (van Riper et al. 2002). A positive correlation was found between increased rainfall, higher mosquito density and the prevalence of avian pox (van Riper et al. 2002). While not necessarily proving that avian pox was the sole cause for population declines as a combination of pressures, including the introduction of mammals has also been identified as leading to population declines, it is now thought that avian pox was (and continues to be) a serious contributing factor to the demise of many Hawaiian avian species (van Riper et al. 2002). The study of Hawaiian birds is especially pertinent for understanding the role of pox in conservation as comparisons were made between both introduced species and endemic species and then related to differences in vulnerability between the two groups.

In the Canary Islands, a study comparing the rates of infection between continental and island native species (Smits et al. 2005) found a very high prevalence of avian pox in island dwelling short-toed larks (*Calandrella*

rufescens) and Berthelot's pipits (*Anthus berthelotti*). Prevalence of the disease was correlated with the presence of poultry farms with stock highly parasitized by fleas suggesting the poultry acted as a reservoir of the disease that then spread to native birds (Smits et al. 2005). On the Galapagos Islands, an investigation on the prevalence of pox in Darwin's finches revealed a relatively high prevalence of avian pox with evidence that the disease has a negative effect on male fitness (Kleindorfer & Dudaniec 2006).

Avian Pox in New Zealand

Avian pox has been reported in New Zealand a number of times in the past. The disease was first observed in poultry (Howse 1949) where considerable losses were reported due to outbreaks in many parts of the North Island in 1948. One of the first observations of avian pox in a wild bird was a report in 1952 in the North Island of a New Zealand pipit (*Anthus novaeseelandiae*) with a single lesion on one leg with another on the face, big enough to cause blindness (Westerkov 1953). Pox lesions were identified in 3% of silvereyes surveyed in the North Island in the late 1960's and early 70's (Austin et al. 1973). In a more recent New Zealand study where molecular techniques were used to isolate avian poxviruses, the disease was identified in as many as 15 different species (Ha et al. 2011).

With regard to management and protection against this disease, currently a number of live vaccines have been developed for protection against

specific strains of avian pox (Tripathy & Reed 2008), and as a matter of caution, fowl pox vaccinations are available for use in the commercial poultry industry in New Zealand. However, several studies on the cross-protection potential of commercial vaccines overseas reveal that they have limited scope to protect against the many variant strains in circulation (e.g. Winterfield & Reed 1985; Fatunmbi & Reed 1995; Singh et al. 2000). Our understanding of the strains that exist in New Zealand, the exact methods of transmission, prevalence in different species, likely danger to threatened species and appropriate management steps for wildlife conservation are limited and require further research.

New Zealand shares many similarities with Hawaii. Like Hawaii it has been geographically isolated for a long period (Cooper & Milliner 1993), it has a proportionately high rate of endemism in its avian species, exotic birds and mammalian predators have recently been introduced, habitat has been greatly modified, and many of its bird populations have experienced severe bottlenecks (e.g. Hale & Briskie 2007). Concern about the negative effect of avian pox on Hawaiian bird populations raises questions about the possible vulnerability to this disease in the endemic bird species of New Zealand. The effects of population bottlenecks is especially considered to be an important issue in New Zealand because there is evidence to suggest that populations with reduced genetic diversity are more vulnerable to diseases such as avian pox and malaria (e.g. Spielman et al. 2004; Hale & Briskie 2007). Detailed information about the prevalence and impact of avian pox on New Zealand bird

species is currently limited and the main objective of my study is to provide more information about this disease.

Avian Pox in Silvereyes (Zosterops lateralis)

The silvereye (*Zosterops lateralis*) is a member of the white-eye (Family Zosteropidae). It is self-introduced, and a relatively new arrival, having only colonized New Zealand in the early 19th century from populations in Tasmania (Clegg et al. 2002). It is likely that silvereyes passed though a population bottleneck during the colonisation process therefore making it a suitable model species to investigate the possible connection between bottlenecks and the negative effects arising from the presence of avian pox viruses. The widespread occurrence of silvereyes also makes them ideal to study the prevalence of avian pox and it effects. Their abundance (silvereyes are now one of the most common passerine birds in native New Zealand forests) may also mean that they play an important role in the general epizootiology of avian pox in New Zealand. Anecdotal reports of pock-like lesions in New Zealand silvereyes from participants of an ongoing, nationwide, annual garden bird survey in 2009, raise the possibility that avian pox may indeed be having an impact on this species (Spurr 2012). It is even possible that declining numbers of silvereyes observed between 2006 and 2009 could be attributed to an epizootic of avian pox.

Outline of Thesis

While this study investigates the prevalence of pox in a range of passerine species it is principally focused on the silvereye with the aim of gaining a more detailed picture of the prevalence and impact that this disease may have on a single species. I examine whether avian pox is a prevalent disease in New Zealand silvereye populations and how the disease compromises the health and condition of individuals.

In Chapter 2, I begin at a broad scale by examining differences in the prevalence of avian pox in a community of passerines surveyed in a native forest near Kaikoura (one of the three locations where silvereyes were also studied). I discuss the results of a direct survey on the presence of pox lesions where comparisons are made between native, endemic and introduced species to determine the extent and pattern of this disease in a local context. In this chapter, I also use the literature to investigate the prevalence of avian pox across a number of passerine species and how it varies across species in terms of degree of residency (e.g. whether migratory, introduced, native or endemic). Analysis of data from the literature is used to gauge vulnerability to avian pox in different species or populations and to test the hypothesis that prevalence of pox should be higher in bird populations that have experienced a bottleneck event.

In chapter 3, I discuss the prevalence of avian pox in silvereye populations surveyed in three separate locations of the South Island between 2010 and 2012. My objective was to determine whether the

prevalence of avian pox was similar across the geographic range of one species. I test whether avian pox is more frequent in the West Coast region due to higher rainfall and increased vector density in this area. I also review the literature review on the relative extent of avian pox in silvereyes compared to other white-eye species overseas.

Chapter 4 examines the hypothesis that pox lesions have a direct effect on the condition of silvereyes and that this can be ascertained through measurement of fat reserves and body mass. I investigate the effects that lesions may have on the morphology of infected individuals. I also explore the impact of this disease through the examination of leucocyte profiles, leucocyte morphology and erythrocyte cell morphology between infected and non-infected silvereyes.

My findings are summarised in the General Discussion where I also put them into context by outlining the implications and importance of avian pox for managing the conservation of endemic bird species in New Zealand. I also make suggestions about much needed research to improve our knowledge about avian diseases for the better preservation of biodiversity in Aotearoa.

Each chapter is in the form of a stand-alone paper in order to facilitate publication and therefore some information is repeated, particularly in the introduction and method sections.

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Chapter 2

Prevalence of avian pox in a sample population of native and introduced species in the South Island of New Zealand

Abstract

In the austral spring (September and October) of 2012, 492 passerine birds were mist-netted in or near Kowhai Bush, Kaikoura, and examined for external signs of avian pox. The biggest proportion (64%) of birds captured was introduced species, mainly from the genera *Carduelis* and *Turdus*. Of the native and endemic species surveyed where silvereyes (*Zosterops lateralis*) accounted for 38.5% of this group, only one case of avian pox was detected and this was in a silvereye (1/69, 1.4%). In the introduced species avian pox lesions were only observed in chaffinches (*Fringilla coelebs*) where 2 cases were identified out of a total of 52 (3.8%) of chaffinches examined. Introduced species may act as a reservoir of avian pox and pose a danger to native bird species but this requires further investigation. A literature review of the worldwide prevalence of pox confirms that there is a significantly higher prevalence of avian pox in island endemic passerine species than in other groups. This may be due to long periods of geographical isolation followed by the introduction of exotic vectors, as well as exotic birds that act as reservoirs of the disease. A number of factors affect the epizootiology of avian pox and I present evidence to suggest that monitoring wild bird populations for this disease should be an important part of conservation practice in New Zealand.

Introduction

Avian pox is a viral disease currently classified in the genus *Avipoxvirus* in the family Poxviridae (Tripathy & Reed 2008). Numerous species of the pox virus afflict many different vertebrate species and avian strains of this disease have been described in a wide range of bird species covering as many as 20 different orders (van Riper & Forrester 2007). Avian pox is distributed worldwide in the commercial poultry industry and has been identified in domestic flocks, pet birds and wild bird populations (Tripathy & Reed 2008). Our understanding of the disease has economic importance because infections lead to loss of production and mortality in domestic poultry (Tripathy & Reed 2008). Recent literature has also drawn attention to the effects of avian pox on endangered wild birds living on geographically isolated islands (e.g. van Riper et al. 2002; Smits et al. 2005; Kleindorfer & Dudaniec, 2006).

Fully developed avian pox viruses are large and brick-shaped, measuring approximately 330 X 280 X 200 nm (Tripathy & Reed 2008). Pox viruses are made up of protein, lipid and double-stranded DNA. Avian pox viruses are hardy and some strains have shown resistance to ether and chloroform (Tripathy & Reed 2008). They can also withstand desiccation and survive in scabs for months or years (Tripathy 1993). Recent use of molecular techniques to investigate the genomic characteristics of viruses has increased our understanding about the differences between

the various strains of avian pox (e.g. Ghildyal 1989; Luschow 2004; Thiel 2005). Analysis of a European strain of fowl pox (FP9) determined that the genome contains a sequence of approximately 260 Kbp (Laidlaw & Skinner 2004). Strains are generally classified according to host specificity and as many as 13 different strains have been recognised as separate species (Tripathy 1993). For the viruses that have been identified with molecular techniques, comparisons with fowlpox show that significant genetic differences exist between canary, quail and mynah pox strains (Tripathy & Reed 2008).

Avian pox is spread through physical transmission where the virus makes direct contact with an abrasion or skin wound (van Riper & Forrester 2007). Biting insects, such as mosquitoes, are known to be vectors as the virus can be carried and transferred from mouthparts (e.g. Akey 1981). The virus can also be transmitted via dust particles from feathers or the dried scabs of infected birds (Tripathy & Reed 2008). Observations of pox infections in domestic birds, has revealed that the incubation period for the disease is between 4 and 10 days (Tripathy & Reed 2008). In wild birds the disease appears to have a longer incubation period, with cases ranging as long as 81 days or up to 13 months in some species (van Riper & Forrester 2007). While the disease can occur at any time of the year avian pox is most common during the months where insect densities are highest and in temperate zones this is most likely in summer and autumn (Tripathy 1993). The epizootiology and prevalence of avian pox in bird populations is influenced by multiple factors and may include the type of

strains in circulation, vector density, host density, host resistance and weather conditions (van Riper & Forester 2007).

Avian pox appears in two main forms. The cutaneous (dry) form leads to the growth of nodular, wart-like lesions caused by rapid multiplication of epithelial cells (hyperplasia) in which infected skin cells also become hypertrophic, affecting skin on the unfeathered parts of a bird, especially the digits, legs, face and eyes (van Riper & Forrester 2007). The disease may persist for up to four weeks in chickens where lesions initially become inflamed only to later hemorrhage and form scabs. Eventually scabs heal over to be replaced by normal skin (van Riper & Forrester 2007). In wild birds, usually only one or two lesions are commonly seen: van Riper & Forrester (2007) suggest that this may be because wild birds have developed a form of natural resistance and that host and parasites have coevolved. It is also possible that few lesions are seen in wild birds as individuals with larger numbers of lesions may not live long. The other form of avian pox is known as diphtheritic or wet pox and is considered to cause greater morbidity than the cutaneous form of pox (Tripathy & Reed 2008). In the wet form of the disease lesions tend to grow in the mouth and mucous membranes of the upper respiratory tract and such infections have resulted in high numbers of fatalities in domestic birds (van Riper & Forrester 2007). This form is not often seen in wild birds, probably because they either die rapidly after infection or they become easy targets for prey (van Riper & Forrester 2007).

Recent studies of endangered endemic island bird species has brought attention to the serious negative impact that avian pox can have on some bird populations and raises questions about the potential for avian pox to have a similar impact on bird species in New Zealand (e.g. van Riper et al. 2002; Smits et al. 2005; Atkinson et al. 2005; Kleindorfer & Dudaniec, 2006). Research in Hawaii provides evidence of a strong link between the importation of avian stock and the introduction of virulent strains of avian pox that ultimately lead to extinctions and a continuing rapid decline in rare endemic bird populations (Jenkins 1989; van Riper et al. 2002, Atkinson et al. 2005). Furthermore, it is suggested that the introduction of the mosquito *Culex quinquefasciatus* has been a major catalyst for successful transmission and spread of the disease in Hawaii (van Riper et al. 2002; Jenkins et al. 1989). Several strains of avian pox have been isolated in mosquitoes and shown to be transmissible from adult insects to birds (e.g. French & Reeves 1954, DaMassa 1966, Akey et al. 1981). Similar losses attributed to the negative effects of avian pox on geographically isolated islands have also occurred on the Canary Islands with endemic short-toed larks (*Calandrella rufescens*) and Berthelot's pipits (*Anthus berthelotti*) (Smits et al. 2005). The negative effect of avian pox on the success of Darwin's finches has also been documented on the Galapagos Islands (Kleindorfer & Dudaniec 2006).

New Zealand shares similarities with Hawaii in that it is geographically isolated and endemism among the birds is very high. Like Hawaii recent introductions of exotic bird species and biting insect vectors such as *C.*

quinquefasciatus to New Zealand have led to the introduction of novel pathogens, for example, avian malaria (*Plasmodium relictum*) (Tomkins & Gleeson 2006). Recent research on the strains of avian pox in New Zealand by Ha et al. (2012) revealed cases of fowlpox in both introduced and native bird species and the detection of two subclades of avian pox including a European strain. This suggests the spread of novel avian pox viruses to New Zealand through the introduction of exotic bird species. Many of New Zealand's native birds have already suffered severe population bottlenecks due to reduced habitat and introduced mammalian predators (e.g. saddleback (*Philesturnus carunculatus*), kokako (*Callaeas cinerea*), yellowhead (*Mohoua ochrocephala*) and black robin (*Petroica traversi*)). Evidence suggests that genetic bottlenecks increase vulnerability to disease (e.g. Altizer et al. 2003; Briskie & Mackintosh 2004; Hawley et al. 2006; Hale & Briskie 2007; Heber & Briskie 2010) and given that at least two strains of avian pox virus are present in bird populations in New Zealand (Ha et al. 2012), the potential may exist for epizootic events in vulnerable bird species. The fact that avian pox was detected in as many as 15 bird species, 10 of which are endemic to New Zealand (Ha et al 2012) suggests the disease is widespread and perhaps even that some species may act as reservoirs and spread the disease to New Zealand's rare avifauna as has occurred in Hawaii.

The objective of this study was to survey a variety of introduced and native bird species to screen for the presence of pox lesions and gauge

the prevalence of avian pox in a specific area of New Zealand. A higher prevalence in any particular species will provide valuable information about the distribution and effects of the disease on New Zealand's avifauna. Assessing the prevalence of avian pox in introduced bird species may provide information about the potential threat of this disease spreading to New Zealand's endangered native bird species. This phase of research is also intended to provide useful comparisons for my other investigation about the prevalence and impact of avian pox in New Zealand silvereyes (*Zosterops lateralis*; see chapters 3 and 4).

In the mid to late 1800's a variety of European birds were introduced into New Zealand (e.g. Long 1981). Many of these introductions were passerine species, including finches, sparrows, accentors, thrushes and starlings. These introductions were of a small to modest number of individuals with releases occurring at different stages (Veltman et al. 1996) and therefore these species were also subject to population bottlenecks. Screening populations of introduced birds alongside their native counterparts for the presence of avian pox and assessing general condition should provide additional information about the health status of introduced populations and the role they may play in the epizootiology of avian pox in New Zealand.

Methods

To assess the prevalence of avian pox in a mixed population of bird species, I screened for the disease in a single location over a period of two months in the spring of 2012 (September and October). The survey was conducted in the South Island of New Zealand in and around Kowhai Bush (42°22'34 S, 173°36'57 E; elevation 72 m), an area located 6 km east of the township of Kaikoura (figure 2.1). Kowhai Bush is an area of mixed regenerating bush largely dominated by manuka trees (*Leptospermum scoparium*). The bush area is within a 4-5 km range of high mountainous terrain and a braided river runs along the southern boundary. Kowhai Bush has been designated as a flood zone and is surrounded by dairy farms creating an area of mixed habitat ideal for catching both introduced and native bird species.

Birds were captured using mist nests ranging in length from 9 m to 17 m. The nets were assembled in different zones each day to increase the probability of catching a wide range of species. To increase the likelihood of netting introduced birds, one 17 m and three 9 m nets were erected in a line on one side or either sides of roads leading towards the Kowhai Bush zone. Birdseed was spread close to the net area to attract finches and other seedeaters. Nets were moved to the edge of Kowhai Bush or placed in small clearings in order to increase the probability of capturing native species. Birdsong recordings were used to attract native species at both roadside and bush settings. Bellbird (*Anthornis melanura*) alarm,

grey warbler (*Gerygone igata*) alarm and song, and brown creeper (*Mohoua novaeseelandiae)* and shining cuckoo (*Chrysococcyx lucidus*) calls were played singularly using an Apple iPod device attached to an Insignia portable NS-MP3CS2 speaker system. At some of the mist netting sites, pinecones smeared with beef fat were placed close to the net to attract silvereyes.

When removed from the nets birds were placed in brown 243 X 202mm disposable paper bags before being weighed and checked for any signs of pox lesions. Double bags were used to contain the larger species, such as song thrushes (*Turdus philomelos*) and blackbirds (*T. merula*). All captured birds were banded in accordance with the regulations stipulated by the New Zealand Government's Department of Conservation. Assessment of condition was achieved through direct measurement of mass using an Ohaus Scout digital scale accurate to 0.1 g and by allocation of a fat score using a 0-5 continuum scale (Krementz & Pendleton 1992). The score was assessed by blowing the contour feathers below the chin to reveal an area where fat is usually stored below the trachea and between the furcula. Next, primary feathers on the left wing were checked for any signs of mites before inspecting the feet, legs, wings and face for any signs of pox. Individuals were classified as infected with avian pox when any swelling was visually detectable as a nodular mass greater than 0.5 mm. Individuals presenting with lesions were photographed, and where relevant, the location, size and the number of lesions were noted. If lesions were identified then a blood

sample was taken and stored in a vial containing 1.5 ml of Queen's lysis buffer solution (Seutin et al. 1991) for later genetic analysis of the strain although the results of these tests are not presented here. A blood smear was also taken for future analysis of white blood cell profiles (see methods in chapter three). Bird handlers were required to wash their hands with alcohol cleanser between handling each bird to minimise the risk of transferring parasites and disease.

To compare the prevalence of avian pox in this investigation with other studies, I reviewed and analysed studies from the literature in which species were surveyed both for the presence or absence of pox, and where prevalence was estimated. In studies where the incidence of pox was recorded according to gender, age, elevation and other variables, I amalgamated these subgroups in order to calculate the percentage of pox for each species. I only used studies conducted on passerine species in which the sample sizes were 20 or more. Each species was classified according to location, time of study and whether it was an introduced, native or endemic species to the study location. I also classified each species as migratory or non-migratory in order to detect any differences in the prevalence of the disease with degree of residency.

Results

A total of 492 birds were caught in and around Kowhai Bush, Kaikoura in the months of September and October 2012. Out of this total, 313 were

introduced species, and apart from 68 silvereyes and one welcome swallow (*Hirundo tahitica neoxena*) which are both self-introduced native species, the rest were identified as endemic to New Zealand.

Cases of avian pox in populations surveyed in Kowhai Bush

Two cases of avian pox (cutaneous form) were observed in chaffinches (*Fringella coelebs*) and these were the only cases in any introduced species (table 2.1). A single pink, nodular lesion measuring approximately 2mm x 2mm was detected on the right side of a male chaffinch's face at the base of the bill (figure 2.2). The pox infection was classified as a "heavy" infection (see chapter 3). This bird also had a small bald patch on its head. Its body mass was 19.1 g, which was the lightest mass recorded out of all the male chaffinches and 2.9 g below the average weight for healthy male chaffinches caught at this location (n = 33). The other chaffinch (female) identified with avian pox presented with a nodular growth between two digits on its right foot (figure 2.3). The lesion's measurement was 3.3mm x 3.3mm and the infection was classified as "light". Its body mass was 22.1 g, which was above the average mean of 20.9 g for females caught that season (n = 15).

Examination for external lesions in native and endemic birds revealed only one case of avian pox in a silvereye (table 2.2). This bird presented with thickening of the skin (1 mm x 2 mm) on the wrist of its right wing, indicating the early stages of a developing lesion (figure 2.8). Another silvereye caught in the spring of 2012 at Kowhai Bush was also identified

with a very light abrasion on the wrist of its left wing (figure 2.7), which may also have been an early sign of avian pox.

A missing toe in one of the bellbirds and one of the redpolls (tables 2.1 and 2.2) may have resulted from a past infection from avian pox as lesions can regress and heal over (van Riper & Forrester 2007). If the degree of prevalence is calculated on the basis of missing claws or missing digits that may indicate an old wound from avian pox then 1.1% of native and endemics showed signs of being affected by this disease. Calculating prevalence in a similar way and including the two cases of avian pox in chaffinches I found that 2.2% of introduced birds showed signs of infection. Overall prevalence of avian pox based on the detection of visible lesions in introduced species was less than 1%. The prevalence of the disease in chaffinches based on the detection of lesions was 3.8%.

The number of lice varied across individuals and species (tables 2.1 and 2.2). Although the sample size of individuals with pox was too small to compare statistically, there did not appear to be any relationship between parasite load (number of lice counted on the wing) and infection with avian pox as chaffinches (the only introduced species with pox) generally had fewer lice than other species with no pox (e.g. greenfinch) and the same pattern was evident between silvereyes (few lice) and other native species (tables 2.1 and 2.2).

Other abnormalities and signs of the disease

While not specifically classified, other external signs of disease and abnormalities were noted in both introduced and endemic species. A female bellbird was observed with a large bald patch on top of its head (figure 2.4) and a male bellbird presented with a healed wound where part of one toe was missing. One male blackbird had inflamed tissue around the eye, while another male blackbird had a deformed beak (slight lateral crossover). The beak deformity may have reduced the bird's ability to preen as it was covered in lice and its feathers were in poor condition (figure 2.5). The bird's mass (95.1 g) was well above average when compared against all males caught in the same area (87.1 g, range 68.9 g - 99.3 g, n = 22). The individual with an eye inflammation had a mass of 82.8 g. Out of the 66 song thrushes captured, one had an area of bare skin between the scapulars below the head. A clump of feathers fell off during capture revealing an extensive area of bare tissue (figures 2.6a & 2.6b). While disease cannot be discounted it appears that this bird has hit the net at speed and torn its skin. The mass of this bird (unsexed) was 64.7 g and was below average when compared to the whole group of song thrushes (69.3 g, range 59.6 g - 84.6 g, n = 64 where mass was obtained for both sexes). Another song thrush was identified with a broken toe that had healed over.

Prevalence of pox in other populations

A review of the literature revealed rates of avian pox ranging from 0 to 50% of a given population (table 2.3). The median score for prevalence in all the passerine species for which I could find information was 6.5 ± 1.04% (SE). When I compared this according to whether species were island endemics, native resident birds, or introduced birds (figure 2.9) I found a significant difference (Kruskal Wallis: H = 18.6, df = 3, P = 0.0003). As some of these measures were the same species, to minimise pseudo-replication, I re-analysed the data using the mean values for each species. I again found significant differences between the four groups of birds (Kruskal Wallis: H = 14.9, df = 3, P = 0.0019). Prevalence of avian pox was highest amongst endemic species compared to other groups (figure 9). The median score for avian pox prevalence in island endemics was 8.85% (n = 28, range, 0-50%). Results for the groups ranged from 1% (n = 22, range, 0-14%) in native species, 1% (n = 17, range, 0-11%) in migratory species, and 1.95% (n = 12, range, 0-21.5%) in introduced species. When the median prevalence score for each residency group is calculated using the mean prevalence within each species (to avoid pseudo-replication) I found that island endemics had a median score of 14.3% (n = 15, range, 0-50%), native species, 1.6% (n = 9, range, 0-9%), migratory species, 1.4% (n = 10, range, 0.09-11%) and introduced species, 1.84% (n = 7, range, 0-21.5%).

Discussion

I found a low incidence of pox in both native and introduced species during the spring and early summer of 2012 in Kowhai Bush, Kaikoura. No differences were detected between species apart from chaffinches where two cases of avian pox were observed and silvereyes where one lesion was observed. A pox lesion was identified on the bill of a male chaffinch while another lesion was observed on the left foot of a female. There were some cases where missing digits had healed over or missing claws were noted in a range of species. While avian lesions can heal and leave such disfigurements none of these old wounds could be verified as attributable to past infections from avian pox. However, if one assumes that some of these may be the results of past infections, then the overall incidence of pox was 1.8% and included also bellbirds (an endemic species) redpolls, goldfinches (*Carduelis carduelis*), dunnocks (*Prunella modularis*), and song thrushes (the latter 4 being introduced species).

The low detection rate of avian pox in the introduced species in this investigation match patterns of avian pox prevalence reported for wild passerine species in continental habitats where prevalence has commonly been documented between 0.5 and 1.5% (van Riper & Forrester 2007). The development of a strong co-evolutionary relationship between host and parasite has been proposed as a likely reason for a lower prevalence of the disease in continental passerine species (van Riper & Forrester 2007). The low incidence of avian pox

among introduced bird populations in this investigation raises the possibility that passerine birds introduced to New Zealand may have retained some immunity to strains of avian pox originating from their native geographical range. To answer this question would require further investigation using molecular techniques to isolate viruses and match them with those classified already.

Visual screening for avian pox revealed avian pox in one silvereye with no sign of the disease in other native or endemic species suggesting that avian pox was not a problem for these populations in the Kowhai Bush area at the time of this study in September and October (spring) 2012. A similar lack of pox was observed in another study investigating avian diseases in endemic birds on Tiritiri Matangi Island, off the coast of the North Island between 2001 and 2004 (Parker et al. 2006). The study included the visual screening for avian pox in the following species; North Island fernbird (*Bowdleria puntata vealeae* n = 25), North Island tomtit (*Petroica macrocephala* n=35), bellbird (*Anthornis melanura* n = 10), tui (*Prosthemadea novaeseelandiae* n = 7), New Zealand robin (*Petroica australis* n = 45) and whiteheads (*Mohoua albicilla* n = 14).

An important point to consider when investigating avian pox is that the prevalence of the disease is influenced by a number of biotic and abiotic variables (van Riper & Forrester 2007). Host immunity, the type of strains circulating in a population, season, temperature, pattern of rainfall and vector densities are just some of the factors that influence the

epizootiology of this disease (van Riper & Forester 2007). In temperate zones it has been observed that avian pox tends to be more prevalent during late summer and autumn (van Riper & Forrester 2007). I was only able to survey avian pox in a range of passerines in spring. It is possible, therefore, that higher infection rates might be observed in other seasons. A summary of the literature on the prevalence of pox in passerine birds (table 2.3), points to a disease where prevalence in a bird population can vary from year to year. For example, avian pox varied from 3% to 14% across 4 years in the golden-headed manakin (*Pipra erythrocephala*; Tikasingh 1982) and 2.4 to 8.2% in the small tree finch (*Camarhynchus parvulus*) across 5 years (Kleindorfer & Dudaniec 2006).

In my study the possibility remains that birds infected with avian pox were unable to make it through the winter where harsh conditions may have reduced the probability of finding sick individuals. In May (autumn) of this year, a colleague mist-netted 14 fantails (*Rhipidura fuliginosa fuliginosa*), in the same area as the present study and one of them had a prominent avian pox lesion. The lesion was located on the hallux of the left leg and measured 2.7 x 2.3 mm (A. Thierry, *pers. comm*). This observation lends weight to the importance of conducting extended investigations in order to detect changes in prevalence of avian pox due to seasonal effects.

One possible sampling bias that may have influenced my ability to accurately assess the prevalence of pox may stem from the disease

compromising a bird's mobility and ability to forage, consequently making them less likely to be captured. Any birds infected with diphtheritic forms probably die quickly so that they are unlikely to be caught at all. Screening juvenile individuals for avian pox may have increased the scope for detecting avian pox, however, such a survey was beyond the scope of this investigation. Indeed, surveying young of the year would be an important area to explore in the future as many studies suggest that juveniles are more vulnerable to avian pox than adults (e.g. Vargus 1987; Buenestado et al. 2004; Senar & Conroy 2004) and this could give a more accurate assessment of prevalence.

Several studies support the contention that there is a tendency for endemic, island dwelling species, to show a higher prevalence of avian pox. The Galapagos, Canary, and Hawaiian Islands are three examples of locations where bird endemism is high, and the studies there reveal increased levels of the disease in many of the bird populations. In Hawaii, infection with avian pox was reported at 34.9% in the native Apapane (*Himatione sanguinea*) and almost 20% or higher in 4 other natives (van Riper et al. 2002). This was in contrast with the lower rates recorded in introduced species (van Riper et al. 2002). The Hawaiian study highlights the increased vulnerability of endemic island birds to avian pox where evidence suggests that geographic isolation and subsequent lack of previous exposure to avipoxviruses and avian malaria are key reasons for extinctions and a recent decline in endemic avifauna. Similar patterns were observed in the Canary Islands (Smits et al. 2005) where it was

found that 50% of short-toed larks (*Calandrella rufescens)* and 28% of Bertholet's pipits (*Anthus berthelotti*), both natives, had lesions symptomatic of avian pox. These species were considered less likely to have been in contact with avipoxviruses than those species with wider ranges where the latter presented with significantly lower rates of infection. Fortunately, at present, high rates of avian pox have not been reported amongst native birds in New Zealand.

The degree to which population bottlenecks and subsequent decreased genetic variation has on predisposing introduced or endemic species to avian pox requires further investigation. Some species have been through bottlenecks that were more severe than others and there are cases in New Zealand where avian pox was the suspected cause of fatalities of birds in severely bottlenecked bird populations, e.g., South Island saddlebacks (*Philisturnus carunculatus* Hale 2008) and Chatham Island black robins (*Petroica traversi* Tisdell & Merten 1988).

While my screening of a community of forest birds in one location of the South Island revealed a low incidence of pox, this does not mean that the risk of avian pox, which other studies have shown can cause serious fatalities in wild bird populations (Jenkins 1989; van Riper et al. 2002, Atkinson et al. 2005) can be ignored. A longer-term study over several years that encompasses a range of seasonal conditions and investigates species that have been exposed to a variety of environmental pressures including severe genetic bottlenecks is required to fully understand the

prevalence and impact of this disease in New Zealand. Any introduction of new strains of avian pox virus, vectors for transmission or increases in vector densities, especially mosquitoes, has the potential to lead to an increase in prevalence in some species with more extensive impacts on endangered species.

Apart from maintaining strict biosecurity measures to prevent alien species entering New Zealand, the regular screening of populations for bird diseases including avian pox should be undertaken along with the identification of viral strains so that conservation authorities can assess the possible threats to rare avian species. For the translocation of rare species, a common practice for the conservation of birds in New Zealand, it would be prudent to gather information about whether avian pox is present in neighbouring populations before transferring birds. Hosts react differently to different strains (Tripathy & Reed 2008) and therefore it is important that strains are also identified to ascertain risk. For example, there are cases where captive wild passerines have come into contact with canary strains of avian pox resulting in high mortality (e.g. Giddens 1971). Although time consuming and expensive (Parker et al 2006), monitoring for avian pox in wild bird populations may avert declines and possibly extinctions of rare species. Recent work by Ha et al. (2012) has led to the isolation of specific strains of avian pox in New Zealand and our increased knowledge about the disease means that screening for pox in wild populations is becoming a more viable proposition. It is important that researchers working in the field report

cases of avian pox and where possible obtain a sample from lesions (see methods in chapter 3) so that we can further understand the epizootiology of this disease both in New Zealand and overseas.

There is much discussion and concern about recent changes in the prevalence of diseases in wildlife (e.g. Daszak et al 2000). Friend et al. (2001) raise concerns about emerging diseases in avifauna worldwide and their review of the literature led them to conclude that in general the occurrence of disease in birds is both variable and difficult to predict. They also conclude that avian pox is occurring with increasing frequency in different groups of birds. My investigation confirms that avian pox is present in at least one of the species of introduced passerines in Kowhai Bush, but I have no reason to believe the other species are immune. My review of the studies conducted in Hawaii, the Galapagos and the Canary Islands also raises questions about whether avian pox has the potential to cause the extinction of some endemic birds in New Zealand. Furthermore, reviews conducted by others on literature about emerging diseases (including avian pox) point to a general lack of perception about just how important diseases are on the survival of birds (Friend et al. 2001). Friend et al. (2001) recommend that we consider the environments that are conducive to disease rather than just focusing on the particular disease itself if we are to better manage disease in the wild. Based on both the literature and the results of my survey, I similarly stress the importance of monitoring avian pox more regularly in New Zealand than has been the case to date, and that a proactive approach is taken towards

understanding the dynamics of this disease. Further research is required to determine the prevalence of avian pox through surveying a variety of bird species over several seasons in different locations. There is a need for a better understanding about which viral strains are in circulation, the degree of pathogenicity of these strains, and whether indeed transmission of avian pox between species is a risk for native and endemic avifauna in New Zealand.

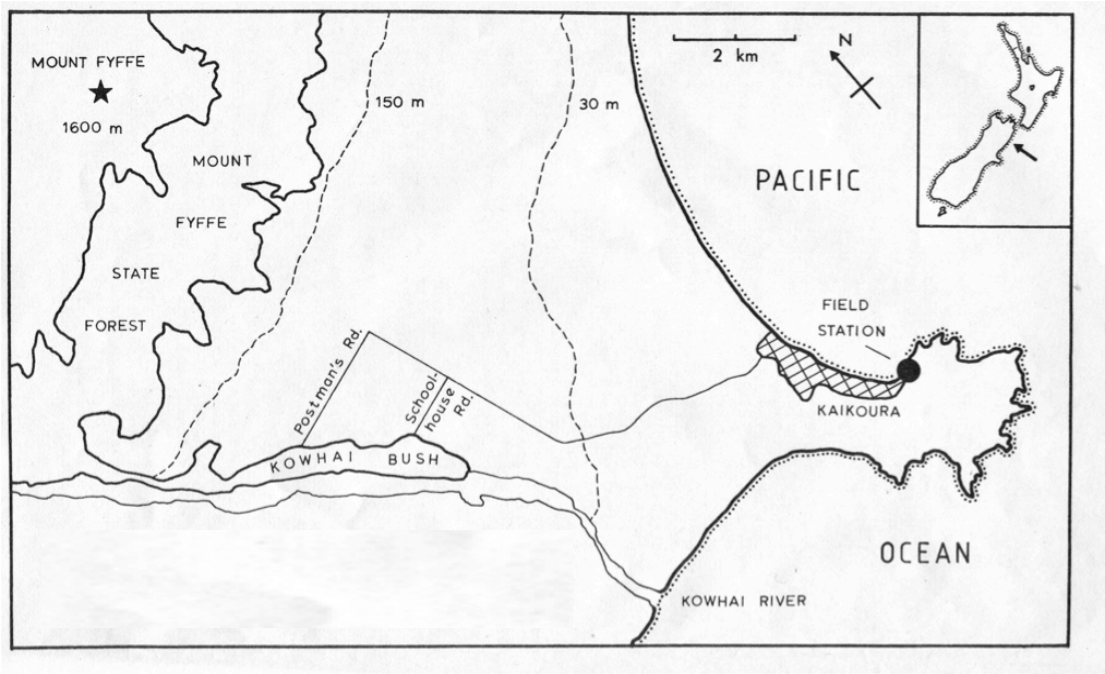

Figure 2.1: Map of the study area in relation to the township of Kaikoura (map from Hunt & Gill 1979). Mist netting was conducted on both Schoolhouse Road and Postman's Road as well as within and around Kowhai Bush.

Figure 2.2: A facial avian pox lesion identified on a male chaffinch (*Fringilla coelebs*) captured at Kowhai Bush, Kaikoura in October 2012.

Figure 2.3: Two nodular lesions growing between the toes on the right leg of a female chaffinch (*Fringilla coelebs*) caught in Kowhai Bush, Kaikoura in September 2012. The largest of the two lesions measured 3mm x 3mm.

Table 2.1: Summary of introduced species captured in or near Kowhai Bush, Kaikoura in the months of September and October 2012. Data is tabulated in columns A-H: A, total number caught; B, number of females identified; C, number of males identified; D, number identified with avian pox lesions; E, number with one or more missing digits, F, number with one or more missing claws; G, average number of lice counted on the left wing; H, number identified with physical symptoms of disease other than avian pox.

Common name	Species	Minimum number introduced (Veltman et al. 1996)	A	B	C	D	E	F	G	H
Chaffinch	*Fringilla coelebs*	449	52	18	34	2	0	0	11 (n=45) SD=23.15	0
Greenfinch	*Carduelis chloris*	65	14	10	4	0	0	0	50 (n=12) SD=37.88	0
Goldfinch	*Carduelis carduelis*	626	76	_	_	0	0	1	6 (n=74) SD=10.22	0
Redpoll	*Carduelis flammea*	607	19	14	3	0	1	0	5 (n=18) SD=9.26	0
Yellowhammer	*Emberiza citrinella*	656	22	7	15	0	0	0	38 (n=18) SD=20.81	0
House sparrow	*Passer domesticus*	416	8	4	4	0	0	0	0	0
Dunnock	*Prunella modularis*	245	18	_	_	0	0	1	0	0
Blackbird	*Turdus merula*	596	35	11	23	0	0	0	5 (n=30) SD=7.8	2
Song thrush	*Turdus philomelos*	343	66	_	_	0	1	1	2 (n=62) SD=6.4	1
Starling	*Sturnus vulgaris*	653	3	_	_	0	0	0	0	0

Table 2.2: Summary of native and endemic species captured in or near Kowhai Bush, Kaikoura in the months of September and October 2012. Data is tabulated in columns A-H: A, total number caught; B, number of females identified; C, number of males identified; D, number identified with avian pox lesions; E, number with one or more missing digits, F, number with one or more missing claws; G, average number of lice counted on the left wing; H, number identified with physical symptoms of disease other than avian pox.

Common name	Species	A	B	C	D	E	F	G	H
Bellbird	*Anthornis melanura*	54	13	41	0	1	0	17 (n=46) SD=22.5	1
Silvereye	*Zosterops lateralis*	69	_	_	1	0	0	1 (n=45) SD=1.76	0
Fantail	*Rhipidura fuliginosa*	8	_	_	0	0	0	3 SD=4.58	0
Grey Warbler	*Gerygone igata*	20	_	_	0	0	0	2 (n=17) SD=3.15	0
Brown Creeper	*Mohoua novaeseelandiae*	7	_	_	0	0	0	39 (n=6) SD=26.4	0
South Island Robin	*Petroica australis*	15	6	8	0	0	0	16 SD=23.06	0
Shining Cuckoo	*Chrysococcyx lucidus*	4	2	_	0	0	0	0	0
Rifleman	*Acanthisitta chloris chloris*	1	1	0	0	0	0	0	0
Welcome Swallow	*Hirundo tahitica subsp. neoxena*	1	_	_	0	0	0	12	0

Figure 2.4: Female bellbird (*Anthornis melanura*) with a bald patch on its crown, caught at Kowhai Bush, Kaikoura, September 2012.

Figure 2.5: A beak deformity observed in a male blackbird (*Turdus merula*), captured in Kowhai Bush, Kaikoura in September 2012.

Figure 2.6A: Bare tissue exposed after a lump of feathers fell off during the capture of a song thrush (*Turdus philomelos*) mist-netted near Kowhai Bush, Kaikoura, September, 2012.

Figure 2.6B: A large clump of feathers that came away from the back of a song thrush (*Turdus philomelos***)**. Note that some of the skin tissue is still attached to the feathers.

Figure 2.7: An area of damaged skin rather than the development of a lesion observed on the wrist region of the left wing of a silvereye (*Zosterops lateralis*), captured at Kowhai Bush, Kaikoura, September 2012.

Figure 2.8: An area of shallow skin thickening, forming a small lesion (1mm x 2mm), on the wrist region of a silvereye (*Zosterops lateralis*) captured in Kowhai Bush, Kaikoura, in September 2012.

Table 2.3: Family and species of Passeridae are listed in cases where prevalence of avian pox has been reported. Data is only included for studied species where n=20 or more. For each study the reported prevalence combines male and female data. Symbols used, (E = Endemic, I = Introduced M = Migrant, N = Native, SI = Self introduced, and, * = Summary of previous data).

Family	Species	Location	Year	Sample size	% with pox	Reference
Pipiridae	Golden-headed Manakin (*Pipra erythrocephala*) (N)	Trinidad	1967 1968 1969 1970	197 399 442 191	14 12 1 3	Tikasingh 1982
	White-bearded Manakin (*Manacus manacus*) (N)		1967 1968 1969 1970	138 386 297 167	0 11 1 4	
	Golden-collared Manakin (*Manacus vitellinus*) (N)	Panama	1967 (October)	103	9	Kirmes & Loftin 1969
Thraupidae	Violaceous euphonia (*Euphonia violacea*) (N)	Trinidad	1968	92	1	Tikasingh 1982
Turdidae	American bare-eyed Thrush (*Turdus nudigeris*) (N)		1968	631	< 0.2	
	Swainson's Thrush (*Hylocichla ustulata*) (M)	Panama	1967 (October)	1,169	10	Kirmes & Loftin 1969
	Gray-cheeked Thrush (*Hyocichla ustulata*) (M)		1967 (October)	436	6	
	Gray-cheeked Thrush	Canada	1965	209	0.5	Kirmes 1966

Table 2.3: (Continued)

Family	Species	Location	Year	Sample size	% with pox	Reference
Turdidae	Swainson's Thrush	Canada	1965	165	< 2	Kirmes 1966
	American Robin (*Turdus migratorius*) (M)		1965	182	1	
	Wood Thrush (*Hyocichla mustelina*) (M)	Panama	1967 (October)	36	11	Kirmes & Loftin 1969
	Veery (*Hylocichla fuscesens*) (M)		1967 (October)	105	2	
	Omao (*Myadestes obscurus*) (E)	Hawaii	1977-80	74	24.3	van Riper & Hansen 2002
	Song Thrush (*Turdus philomelos*) (N)	Czech Republic	2005	45	0	Kulich et al. 2008
	Blackbird (*Turdus merula*) (N)		2005	26	0	
Tyrannidae	Trail's Flycatcher (*Empidonax traillii*) (M)	Panama	1967 (October)	280	< 0.4	Kirmes & Loftin 1969
Parulidae	Mourning Warbler (*Oporonis philadelphia*) (M)		1967 (October)	56	1.8	
	Ovenbird (*Seiurus aurocapillus*) (M)		1967 (October)	90	1	
Emberizidae	Thick-billed Seed Finch (*Oryzoborus funereus*) (N)		1967 (October)	63	1.6	
	Variable Seedeater (*Sorophila aurita*) (N)		1967 (October)	136	< 1	
	Blue-black Grosbeak (*Cyanocompsa cyanoides*) (N)		1967 (October)	25	4	
Petroicidae	Tomtit (*Petroica macrocephala)* (E)	Tiritiri Maitangi Island, New Zealand	2001-04	35	0	Parker et al. 2006
	North Island Robin (*Petroica australis longpipes*) (E)		2001-04	45	0	

Table 2.3: (Continued)

Family	Species	Location	Year	Sample size	% with pox	Reference
Emberizidae	Field Sparrow (*Spizella pussilla*) (N,M)	Panama	1967 (October)	115	< 1	Kirmes & Loftin 1969
	Dark-eyed Junco (*Junco hyemalis*) (N,M)	Canada	1965	608	< 0.5	Kirmes 1966
	Song Sparrow (*Melospiza melodia*) (N,M)		1965	155	< 1	
	Chipping Sparrow (*Spizella passerina*) (N,M)	U.S.A.	1922	287	25	Musselmann 1928
	Chipping Sparrow		1923	563	42	
	Small Ground Finch (*Geospiza fuliginosa*) (E)	Galapagos Islands	2000	89	1	Kleindorfer & Dudaniec 2006
	Small Ground Finch		2001	128	3	
	Small Ground Finch		2002	71	5.6	
	Small Ground Finch		2004	63	9.5	
	Small Ground Finch		2000-04*	351	4.3	
	Medium Ground Finch (*Geospiza fortis*) (E)		2000-04	58	13.8	
	Large Ground Finch (*Geospiza magnirostris*) (E)		2000-04	21	14.3	
	Small Tree Finch (*Camarhynchus parvulus*) (E)		2000	26	7.7	
	Small Tree Finch		2001	41	2.4	
	Small Tree Finch		2002	49	8.2	
	Small Tree Finch		2004	26	3.8	
	Small Tree Finch		2000-04*	142	5.6	
	Warbler Finch (*Certhidea olivacea*) (E)		2000-04	64	4.7	

Table 2.3: (Continued)

Family	Species	Location	Year	Sample size	% with pox	Reference
Motacillidae	Lesser Short-toed Lark (*Calandrella rufescens*) (E)	Canary Islands	2002-03	395	50	Smits et al. 2005
	Berthelot's Pipit (*Anthus berthelotti*) (E)		2002-03	139	28	
Passeridae	Spanish Sparrow (*Passer hispaniolensis*) (N)		2002-03	128	0	
	House Sparrow (*Passer domesticus)* (I)	Hawaii	1977-80	81	7.4	van Riper & Hansen 2002
Fringillidae	Trumpeter Finch (*Bucanetes githagineus amantum*) (E)	Canary Islands	2002-03	228	0	Smits et al. 2005
	House Finch (*Carpodacus mexicanus*) (E)	California, U.S.A.	1972-73	42	17	Power & Human 1976
	House Finch (I)	Hawaii	1977-80	79	21.5	van Riper & Hansen 2002
	American Goldfinch (*Carduelis tristis*) (M)	Alabama, U.S.A.	1999	58	0	McGraw et al. 2001
	Serin (*Serinus serinus*) (N)	Spain	1996	1408	4.4	Senar & Conroy 2004
Mimidae	Galapagos Mockingbird (*Nesomimus parvulus*) (E)	Galapagos, Isla Santa Cruz	1979-80	67 (adults) 50 (Juveniles)	16	Vargus 1987
Certhiidae	Brown Creeper (*Certhia familiaris americanum*) (M)	Ontario, Canada	1965	1079	< 0.1	Kirmes 1966
Zosteropidae	Silver-eye (*Zosterops lateralis*) (N,SI)	New Zealand	1965	95	3	Austin et al. 1973
	Silver-eye (N,SI)		1966-67	957	0	
	Silver-eye (N,SI)		1968-71	>1000	< 1	
	Silver-eye (N,SI)		2010	178	< 2	Taylor 2012 (unpublished)
	Silver-eye (N,SI)		2011	162	3.7	
	Japanese White-eye (*Zosterops japonicus*) (I)	Hawaii	1995	68	0	Atkinson et al. 2005
	Japanese White-eye		1977-80	1,243	2.2	van Riper & Hansen 2002

Table 2.3: (Continued)

Family	Species	location	Year	Sample size	% with pox	Reference
Drepanidinae	Hawaii *Amakihi* *(Hemignathus virens*) (E)	Hawaii	1995	212	3.3	Atkinson et al. 2005
	Hawaii Amakihi		1977-80	626	17.6	van Riper & Hansen 2002
	Iiwi (*Vestiaria coccinea*) (E)		1995	41	0	Atkinson et al. 2005
	Iiwi		1977-80	107	17	van Riper & Hansen 2002
	Apapane (*Himatione sanguinea*) (E)		1995	117	12	Atkinson et al. 2005
	Apapane		1977-80	601	35	van Riper & Hansen 2002
Icteridae	Brown-headed Cowbird (*Molothrus ater*) (M)	Alabama, U.S.A.	1960-61	8,285	0.9	Stewart 1963
Monarchidae	Elepaio (*Chasiempis sandwichensis*) (E)	Hawaii	1977-80	77	19.5	van Riper & Hansen 2002
Estrildidae	Spotted Munia (*Lonchura punctulata*) (I)		1977-80	137	0	
Cardinalidae	Northern Cardinal (*Cardinalis cardinalis*) (I)		1977-80	51	2	
Sylviidae	Red-billed Leiothrix (*Leiothrix lutea*) (I)		1977-80	46	0	
	New Zealand Fernbird (*Bowdleria punctata vealeae*) (E)	Tiritiri Maitangi Island, New Zealand	2001-04	25	0	Parker et al. 2006
	Eurasian Blackcap (*Sylvia atricapilla*) (M)	Czech Republic	2005	244	4	Kulich et al. 2008
Prunellidae	Dunnock (*Prunella modularis*) (N)		2005	36	5.5	
Phyloscopidae	Chiffchaff (*Phylloscopus collybita*) (M)		2005	39	0	
Aegithalidae	Long-tailed tit (*Aegithalos caudatus*) (N)		2005	23	0	
Muscicapidae	European Pied Flycatcher (*Ficedula hypoleuca*) (M)		2005	23	0	
Paridae	Blue Tit (*Parus caeruleus*) (N)		2005	24	0	
	Great Tit (*Parus major*) (N)		2005	44	0	
	Great Tit	Hungary	2007	1819	< 1	Palade et al. 2008

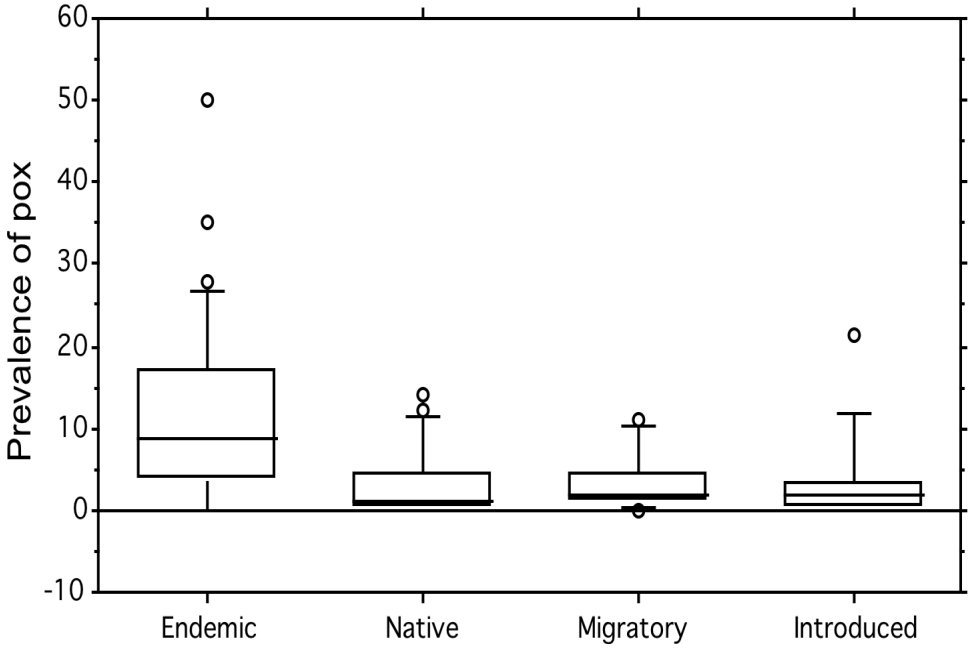

Figure 2.9: Box plot showing the prevalence of pox in passerine species grouped according to degrees of residency. Information was obtained from worldwide literature (see table 2.3) where the prevalence of pox was quantified ($n \geq 20$).

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Chapter 3

Prevalence of avian pox in the New Zealand silvereye (*Zosterops lateralis*)

Abstract

Between June 2010 and October 2012, 409 silvereyes (*Zosterops lateralis*) were captured in Christchurch, Moana and Kaikoura (all locations in the South Island of New Zealand) and assessed for the presence of avian pox lesions. Lesions were identified in 9/409 birds (2.2%), and were found in both adult and juvenile birds, and both sexes. Prevalence for the above locations ranged from 0% to 5% in 2010, and from 1.8 to 7.2% in 2011. When silvereyes were resurveyed in Kaikoura in the spring of 2012 only1/69 (1.4%) of individuals showed signs of avian pox. The results suggest that prevalence rates change over time and vary geographically. The highest prevalence was detected in Moana, a location on the west coast of the South Island with higher rainfall and potentially more insect vectors. Lesions measured from 1 mm up to 6.6 mm in diameter and were most frequently located on the legs and toes. A review of the literature revealed a similar prevalence of avian pox in silvereyes seen in this survey to that observed in other populations in New Zealand and in other white-eye species (Family Zosteropidae) elsewhere. The presence of avian pox in silvereyes, which are abundant and widespread, raises questions about the potential for this species to act as a reservoir and threaten rare native species that share similar environments.

Introduction

Avian pox was one of the earliest diseases to be described in birds (van Riper & Forester 2007). It is easily recognised, and perhaps as a result has been observed in 20 different avian orders and more than 278 species (van Riper & Forrester 2007). Avian pox is a worldwide problem for domestic birds reared on commercial farms and live vaccines have been developed to help with management of the disease (Tripathy & Reed 2008). More recently, outbreaks of the more pathogenic diphtheritic form of avian pox have been observed in chicken flocks previously vaccinated against fowlpox (FPV) (Tripathy & Reed 2008). In wild bird populations where the cutaneous form of pox is seen most often (van Riper & Forrester 2007) there has been a tendency for avian pox to be reported where scientists have been working on the disease and this has created a geographic skew on our knowledge of the disease's distribution (van Riper & Forrester 2007).

The prevalence and distribution of the disease in bird populations is influenced mainly by the strains and numbers of pox viruses in circulation, host density, vector density and weather conditions (van Riper & Forrester 2007). Recent studies indicate that avian pox is most prevalent in temperate or warm climate zones (van Riper & Forrester 2007). It has been suggested that avian pox is more prevalent in autumn and early winter in temperate zones because this is when vector densities

are at a peak, there are more juveniles present and flocking behaviour increases during this time (van Riper & Forester 2007). Avian pox has also been reported with high prevalence in endemic wild bird populations living in geographically isolated islands where exotic avian species have been introduced and where vectors (usually biting arthropods) are present in high densities, for example Hawaii (van Riper et al. 2002; Jenkins et al. 1989; Atkinson et al. 2005), Galapagos Islands (Kleindorfur & Dudaniec 2006; Vargus 1987), and Canary islands (Smits et al. 2005, Illera et al. 2008) (see chapter 2).

There is evidence to suggest that prevalence of avian pox in a given population of birds can change between seasons and years. In native Hawaiian birds an increase in active lesions were noted during July and September while the disease was less prevalent from January through to June (van Riper et al 2002). Tikasingh (1982) observed variation in prevalence of this disease from 3-14% in golden-headed manakins (*Pipra erythrocephala*) over a period of 4 years and a similar fluctuation in prevalence was observed in a 5-year study of small tree finches (*Camarhynchus parvulus*) in the Galapagos Islands (Kleindorfer & Dudaniec 2006).

The presence of avian pox in New Zealand was first documented more than 60 years ago (e.g. Howes 1949; Westerkov 1953; Austin et al. 1973). Early workers relied on surveying birds for outward symptoms of pox but more recent studies have combined this with molecular methods to

confirm the identity of avian pox. Genetic evidence for the presence of avian pox was recently found in as many as 15 avian species, 10 of which are endemic to New Zealand (Ha et al. 2011). While it is clear that avian pox is present in New Zealand and can infect native birds, there is still a gap in our knowledge about the distribution and prevalence of this disease in wild bird populations.

Studies of disease in native birds in Hawaii strongly suggest that the introduction of avian pox led to the demise of many of its endemic species (van Riper et al. 2002). The release of the ornithophilic mosquito (*Culex quinquefasciatus*), regarded as a vector for avian pox, and the introduction of exotic species of birds which serve as reservoirs for the disease, are viewed as the principle reason for the spread of avian pox throughout Hawaii. The previously geographically isolated bird populations in Hawaii had limited immune defenses against avian pox and as a result, more than half of Hawaiian bird species are now extinct or classified as critically endangered (Jenkins et al. 1989; van Riper et al.2002; Atkinson et al. 2005). The apparent vulnerability of insular avian species to pox viruses is given greater credence with evidence that Hawaiian birds have significantly higher rates of pox infection than their introduced counterparts (van Riper et al. 2002).

Research on the effect of avian pox on Hawaiian forest birds raises questions about the possible effects of this disease on the New Zealand avifauna. There is a good case for conducting similar studies on the

epizootiology and effects of the disease here. Like Hawaii, New Zealand has experienced a long period of geographical isolation. Endemic species in New Zealand have also been forced to adapt to the pressures exerted by recently introduced avian exotics, which could likewise introduce new strains of pox and act as reservoirs for the disease. In light of the effect of avian pox in Hawaii, it is critical to determine the risk posed to native New Zealand birds, especially considering many species currently already face immense pressure from introduced mammalian predators, population bottlenecks and reduced habitat (e.g. Holdaway 1991). Thus the objective of this study was to investigate the prevalence of avian pox in one species of New Zealand bird, the silvereye (*Zosterops lateralis).* The New Zealand population of the silvereye is a recent coloniser from a Tasmanian race of this species. Although it is a recent arrival in New Zealand, it nonetheless provides an ideal model species for studying the distribution and prevalence of the disease. Silvereyes are abundant, widespread, and easy to capture. They have passed through a population bottleneck during their colonisation of New Zealand mimicking the population processes experienced by many native species currently subject to bottlenecks and the potential loss of genetic variation. Gathering information about this disease in the silvereye is important as it can provide the basis for understanding the impact of avian pox on rarer species.

Methods

Silvereyes were captured using mist-nets at three locations in the South Island of New Zealand (figure 3.1). Lures made from pinecones laced with a mixture of beef-fat and sugar, were hung within 3 m of the net in order to attract birds to the area. Occasionally apples were used as additional bait. Nets were placed close to trees and set from ground level. Regular monitoring of the net by an observer minimised the time captured birds were ensnared. The study areas included the suburb of Westmorland in the city of Christchurch, Canterbury (43°34'59 S, 172°36' E; elevation 90 m), Kowhai Bush, Kaikoura (42°22'34 S, 173°36'57 E; elevation 72 m) and Moana, Lake Brunner, West Coast (42°34'29 S, 171°28'39 E; elevation 112 m (figure 3.1). The locations were selected to cover a range of habitat and climates but also distant enough to ensure the survey of separate populations. Westmorland is situated on the western side of the Port Hills and the suburban garden habitat is comprised of a variety of native trees and shrubs but also many exotics and fruit trees. Christchurch has a temperate, dry climate with a mean temperature of 12.1°C and a mean annual rainfall of 648 mm (NIWA). Kowhai bush is situated in a designated flood zone, away from urban areas and located alongside a medium-sized braided river. A large area of regenerating young forest, mainly manuka trees (*Leptospermum scoparium*) make up the study area, however, the forest is surrounded by dairy farms. The climate is slightly milder in the Kaikoura region with a higher mean

annual rainfall of 844mm. Moana is located in the West Coast province and is surrounded by mountains and vast tracts of natural rain forest. Moana was chosen as a study site as it experiences significantly higher levels of rainfall than those in eastern regions. No official recordings are available for the immediate area, however the town of Hokitika to the south has an annual mean rainfall of 2875mm and this is similar to that in Westport (2274mm) to the north. These three diverse sites were selected as it was thought that differences in the type of habitat might reveal differences in infection rates possibly due to the increased presence of vectors in areas with higher rainfall. There are 7 known endemic and 2 introduced species of mosquito found on the west coast region of the South Island, including *Culex quinquefasciatus* (Southern Monitoring Services Ltd), a suspected vector of bird pox and avian malaria (van Riper et al. 2002). Many potential vectors, including sand flies (genus *Austrosimulium*) are noticeably present in greater numbers in the wetter, western region of the South Island (pers. obs.).

Once removed from mist nets, birds were placed in small cloth bags and weighed on scales accurate to 0.1g. All birds were fitted with A size bands issued by the New Zealand Department of Conservation. Digital calipers were used to measure the length of the beak (from nares to tip), tarsus and wing chord (not flattened). Fat scores were recorded on a 0-5 continuum (Krementz & Pendleton 1992). Fat measurements were estimated by blowing on the contour feathers under the chin to expose any fat deposits stored just below the skin. Birds were surveyed carefully

for any visible signs of pox lesions (figures 3.2 & 3.3). Individuals were classified as infected when any swelling was visually detectable as a nodular mass greater than 0.5 mm. Silvereyes presenting with lesions were photographed, and where relevant, the location, size and the number of lesions were noted. Classification of infections followed those methods used in a previous study of avian pox in passerine species (van Riper et al. 2002). Infections were recorded as 'light' if only one lesion could be seen, 'moderate' if there were two lesions and heavy when three or more lesions were noted or lesions were found on the head. Figures 2 and 3 illustrate some examples of lesions that I identified as cases of avian pox in the silvereye.

Whenever practicable, blood samples were obtained for later analyses. One of the infected silvereyes captured in Westmorland in 2010 escaped from the mist-net, and although pox lesions were clearly observed and the individual is included in the data set, no measurements or blood samples were taken. To obtain blood one wing was gently extended to expose the brachial vein and a 0.33 X 13mm needle was used to make a small puncture in the brachial vein to remove ~60 μL blood with the aid of a heparinised capillary tube. Blood smears were taken for each captured silvereye using microscope slides (see next chapter) and any remaining blood was placed in a 1.7 ml microtube containing a 1.5 ml solution of Queen's lysis buffer (Seutin et al. 1991). In the case of infected individuals, if lesions were large enough, a scraping was taken using a scalpel blade. Latex gloves and alcohol hand wash were used in order to

minimise the risk of further contaminating birds or materials with virus particles. In two cases where the scraping of larger lesions was considered likely to injure the bird, an aspiration biopsy procedure with a fine needle was used to obtain tissue from inside lesions. A larger 25 gauge needle and syringe allowed retrieval of a small tissue sample using techniques described by Campbell (1994). Samples were placed in glass vials and stored at -20°C (These samples were collected for the analysis of pox strains, but the results of this analysis will be reported later).

Silvereyes are difficult to sex reliably while in the hand; therefore molecular sexing was used as a means of assigning gender (figure 3.4). DNA was extracted from blood using a SIGMA, XNAB2 kit. Blood volumes of 10 μL were added to an equal amount of lysis then left to stand for five minutes, before adding 90 μL of blood neutralization solution. Samples were covered with foil and refrigerated at 4°C. Polymerase chain reactions (PCR) were performed using a mix ratio of 5 μL molecular water, 1 μL of primer (avian sex primers 2550F and 2718R), and 10 μL of the enzyme Kapa A, blood mix B (KapaBiosystems KM7005). Samples were run on an Eppendorf thermal cycler with the same heating and cooling profile as recommended by Fridolfsson & Ellegreen (1999). PCR product separation was achieved with a 1.5% agarose gel mix and SYBR safe DNA gel stain was used for visualisation. All electrophoresis was carried out for a period of 30 minutes at a setting of 120 volts.

To compare the prevalence of avian pox discovered in this study with other studies of avian pox, I conducted a review of the literature on the

prevalence of pox in other populations of silvereyes as well as other species in the Family Zosteropidae ('white-eyes'). I used studies in which species were surveyed both for the presence or absence of pox, and for estimates of the prevalence when present. In studies where the incidence of pox was recorded according to gender, age, elevation and other variables, I amalgamated these subgroups in order to calculate the percentage of pox for each species. I only used studies in which the sample sizes were 20 or more (table 2.3).

Appropriate permits for the capture, banding and blood sampling were obtained from the New Zealand Department of Conservation. Approval was also granted from the University of Canterbury's Animal Ethics Committee before capturing and handling silvereyes.

Results

Prevalence of avian pox

A total of 409 silvereyes were caught at the three locations between 2010 and 2012. Of the 340 silvereyes caught between 2010 and 2011 when blood was taken for sexing, half of this total was caught in the winter and early spring of 2010 while the others were captured in the following year, during the summer and early winter of 2011. Molecular sexing was successful in 287 cases but the gender could not be determined for the other 53, either due to the absence of a blood sample or a failed PCR reaction.

A total of 10 cases (2.4%) of avian pox were detected using visual symptoms to identify pox lesions (see methods). This includes one case of a juvenile in Christchurch captured by a local resident, suggesting the disease can affect both young and adult birds. Sex was determined in 7 out of 9 infected adult birds and 5 of these were females. The sample is too small to statistically test if one sex is more prone to infection than the other, but nonetheless confirms the disease can affect both males and females. The greatest prevalence of pox was observed in Moana with 7.2% (n = 55) of the silvereyes in this region showing signs of the disease between late May and mid-June 2011 (figure 3.5). None of the birds captured in Kaikoura during winter (n = 60, July 2010) presented with lesions and neither did any captured in Moana during the spring (n = 60, late August to early September) of 2010. The Westmorland sample exhibited the second highest infection rate with 5% (n = 58) of individuals presenting with lesions in the winter of 2010. While the greatest number of pox cases were observed in Moana during winter 2011, there were only two infected individuals caught at the other locations that same year, one in Christchurch (n = 53) and the other in Kaikoura (n = 54). None of the silvereyes captured in the spring of 2012 at Kaikoura (n = 69) showed any sign of external pox lesions (figure 3.5). . When compared across the 3 sites, the prevalence of avian pox was significantly different in 2010 (Fisher's exact test: $P = 0.033$) but not in 2011 (Fisher's exact test: $P = 0.37$), confirming the variable nature of prevalence with time and geographic location.

Distribution of pox lesions on individual birds

Lesions were observed on the wings, legs, toes, and in one case on the cornea. The size of lesions ranged from relatively small (1mm x 1mm) to large, with one smooth circular lesion measuring 6.6 mm in diameter (table 3.1). In most cases infections were light or moderate (see definitions in methods). In the two cases of heavy infection, one individual had lesions on every digit of the right foot, including a partly missing digit, while the other individual had a large brown and white, flat growth on the left eye (figure 3.6). The most common parts of the body bearing pox lesions were the legs and feet. Out of the total nine observed cases, seven individuals had lesions in the leg region only. In the other two cases one had a single lesion on the left eye and the other had two lesions on one wing.

For silvereyes, the rates of prevalence I observed were similar to those reported in other studies of this species and of other species of white-eyes (i.e. *Zosterops* spp.), both in New Zealand and Hawaii. In Hawaii between 1977-80, pox lesions were identified in just over 2% of Japanese silvereyes (van Riper & Hansen 2002). A study conducted in the North Island of New Zealand between 1965 and 1971 revealed pox cases in 3% of silvereyes (Austin et al. 1973). In my investigation of avian pox in silvereyes in the South Island, I found that the overall rate of infection for the entire group surveyed was < 2% in 2010, 3.7% in 2011 and 1.5% in 2012. The overall prevalence over the three-year period was 2.4%.

Discussion

The prevalence of avian pox lesions in silvereyes in the South Island of New Zealand was found to be relatively low and comparable to that seen in other populations of this and closely related species. The results of this survey also showed that rates vary geographically and over time. The disease appears to affect both males and females, and is most frequently manifested externally by the growth of lesions on the legs and toes.

The beginning of my investigation coincided with the release of results from annual bird surveys indicating a decline in silvereye numbers between 2007 and 2009 (Spurr 2012). The survey requires volunteer observers to record the highest number of any one particular species seen at one time in the months of June and July. The drop from a national average sighting of 10.2 silvereyes per garden in 2007 to 6.3 in 2009 could be explained by an epizootic, such as avian pox. Indeed, survey organisers received anecdotal reports about wart-like growths on the legs of some of the silvereyes observed in gardens during the years of lower counts (Spurr 2012). My quantitative data on the prevalence of the disease in silvereyes following the reported declines in 2009 indicates that it is still present, but at rates that are comparable to other studies of this species both here in New Zealand and of congeners overseas. Thus, it is not clear if pox was responsible for the reported decline in silvereye

numbers, or if it was, whether the prevalence had decreased by the time of my survey.

I found that infection rates were relatively low in the mid region of the South Island (Canterbury and Westland): < 2% for the combined locations surveyed in 2010, and slightly higher at 3.7% in 2011. Garden bird surveys in 2010 indicated an increase in silvereye numbers for 2010 and this coincided with my low detection rates for pox lesions that same year. It is possible that the low prevalence of avian pox in my study (particularly in 2010) could be attributed to a possible recovery from a previous epizootic phase of the disease, with weaker individuals succumbing to the virus in the previous season leaving healthier survivors. My survey was conducted over a relatively short time frame of two years and therefore does not allow me to confirm the purported cyclic nature of this disease (van Riper & Forester 2007). It would be useful to continue my surveys at regular intervals over the next few years as epizootics have been shown to appear at different seasons or in different years in other studies (van Riper & Forester 2007). Although I surveyed the prevalence of pox over 3 years at Kaikoura, I was unable to cover all the seasons and thus may have missed any seasonal fluctuations in prevalence of avian pox. While a variety of possibilities could explain the earlier declines in silvereye numbers indicated by garden bird surveys (Spurr 2012), avian pox cannot be ruled out as a key contributing factor given the fact that we know it is present in some of the South

Island's silvereye populations and that the disease has significantly impacted other native birds overseas.

The relatively low rate of avian pox infection detected in my study, while comparable with the results of other studies involving silvereyes, does not diminish the need to consider the important implications of the presence of such a disease in populations in New Zealand. Evidence suggests that given the right weather conditions, host density, and an increased presence of suitable vectors (van Riper & Forrester 2007), this disease has the potential to become widespread. Whether this is true for silvereye populations in New Zealand requires longer-term investigations in order to gauge the effects on populations when epizootics do occur.

Research indicates that when vector numbers increase, avian pox viruses are more likely to spread (Forrester 1991; van Riper et al. 2002). I hypothesized that high vector densities on the west coast of the South Island would mean that this area would be the most likely location where silvereye populations would be found to act as hosts for avian pox. While the 2010 survey revealed no evidence of avian pox in Moana, I did observe that 7.2% of silvereyes had lesions there in 2011. Furthermore, the Moana population surveyed in 2011 had the highest incidence of infection when compared with all the other locations throughout the two-year survey. As I was only able to survey one site, I cannot conclude the proposed link between the prevalence of pox and vector density. A higher prevalence of pox could be due to higher vector densities at Moana than

at other locations. As many as 7 native species of mosquito and 2 introduced species are known to be established on the west coast of the South Island (Southern Monitoring Services Ltd), where the wet conditions provide ideal breeding conditions. Sandflies (genus *Austrosimulium*) are also present in very high numbers in this region. While the introduced mosquito *Culex quinquefasciatus* is a suspected carrier of avian pox (van Riper et al. 2002; Whiteman et al. 2005), little is known about the capacity of the other endemic mosquitoes to act as vectors for strains of avian pox. Understanding the life histories of these mosquitoes and which species have the potential to transmit this disease to avifauna is certainly an area worthy of further investigation.

Why lesions are mostly found on the legs and toes of silvereyes is not fully understood. It may be that because the skin of the legs is the most exposed unfeathered part of the body that this is the most likely area to be bitten by mosquitoes. It could also be that the virus is more likely to be picked up on the feet from perches where the virus has been deposited by other infected birds, a phenomenon observed in aviary environments (e.g. Bleitz 1958). The point of entry where the virus is introduced may mean that disease manifests itself there directly in the form of lesions rather than spreading to other parts of the bird's skin. While taking care to inspect individuals carefully for lesions, none-the-less there may be a bias for detection of lesions on the legs when other lesions may in fact be hidden beneath feathered parts of a bird.

Implications for New Zealand

Currently, the role of avian pox in the extinction and decline of native and endemic birds is poorly known. There is clear evidence that introduced mammalian predators have been the key driver of species extinction in New Zealand (e.g. Holdaway 1999), but the role of disease is less certain. An important factor in assessing the added risk from pathogens, such as avian pox, is whether or not native species have sufficient immune responses to counter disease. This will depend on prior exposure (over evolutionary time) and it may also depend on whether there is enough genetic variation present in an extant population to develop immune defences should prevalence increase. Of particular concern is the fact that a large number of native species in New Zealand have already undergone severe population bottlenecks due to rapid declines resulting from predation and loss of habitat (Briskie & Makintosh 2004). One of the effects of a population bottleneck is reduced heterozygosity and genetic diversity (e.g. Frankham 1998) and the consequence of reduced resistance to disease (e.g. Spielman et al. 2004; Hale & Briskie 2007). Many bottlenecked native species have been translocated to predator free offshore or mainland islands. In some cases where translocation numbers are small and genetic heterozygosity has been reduced, e.g. South Island saddleback (*Philesturnus carunculatus carunculatus*), avian pox has been identified as a problem (Hale 2008). A more extreme bottleneck example is the critically endangered Chatham Island black Robin (*Petroica traversi*) where avian pox has also been identified as a significant problem. Mortality has been observed in black robin fledglings

within two weeks of the appearance of pox lesions (Tisdell & Merten 1988).

A possible sampling bias may have influenced my ability to accurately assess avian pox cases. It may be that individuals severely debilitated by lesions were less likely to be caught. In diphtheritic pox, where consequences are known to be severe (Tripathy & Reed 2008), it is unlikely that mist-nets would lead to capture. If the suggested bias is correct then it is likely that avian pox is even more prevalent than studies indicate.

Future work should take into account the need to run a survey for a greater period than two years in order to detect any significant fluctuations in disease manifestation or epizootic events. My review of the literature revealed that there is a pattern of variability in the prevalence of avian pox within a particular species from one year to another. Thus, it is important that future surveys of avian pox are carried out over a number of seasons between years rather than over a single year.

A better understanding of avian pox in silvereyes may result from comparisons with Tasmanian populations. Comparing genetic differences may reveal lower heterogeneity in New Zealand populations. Detection and comparison with any avipoxvirus strains that may exist in Australian

silvereye populations may reveal important information about the source and virulence of this disease.

I suggest that more comprehensive investigations about the effect of avian pox on New Zealand's indigenous avifauna is needed in order to ascertain whether outbreaks similar to those seen in Hawaii and the Canary Islands are likely to impact endemic populations. Given the evidence that avian pox is present in silvereye populations then further investigations are required in order to determine the temporal pattern of this disease as well as the degree of host specificity for any avian pox virus strains that can be identified. Silvereyes are common and widespread in New Zealand and populations may play a role as reservoirs with the potential to directly or indirectly, transmit and spread avian pox viruses to other bird species. Motive for a more in-depth investigation of this disease stems from evidence that the introduction of avipoxviruses to other species situated in geographically isolated areas has contributed to extinction and serious declines of endemic birds (van Riper 2002; Smits et al. 2005; Kleindorfur & Dudaniec 2006). Knowledge about the type of strains, understanding the distribution, host range, etiology and epizootiology of avipoxviruses in New Zealand should be an important part of our conservation and science research.

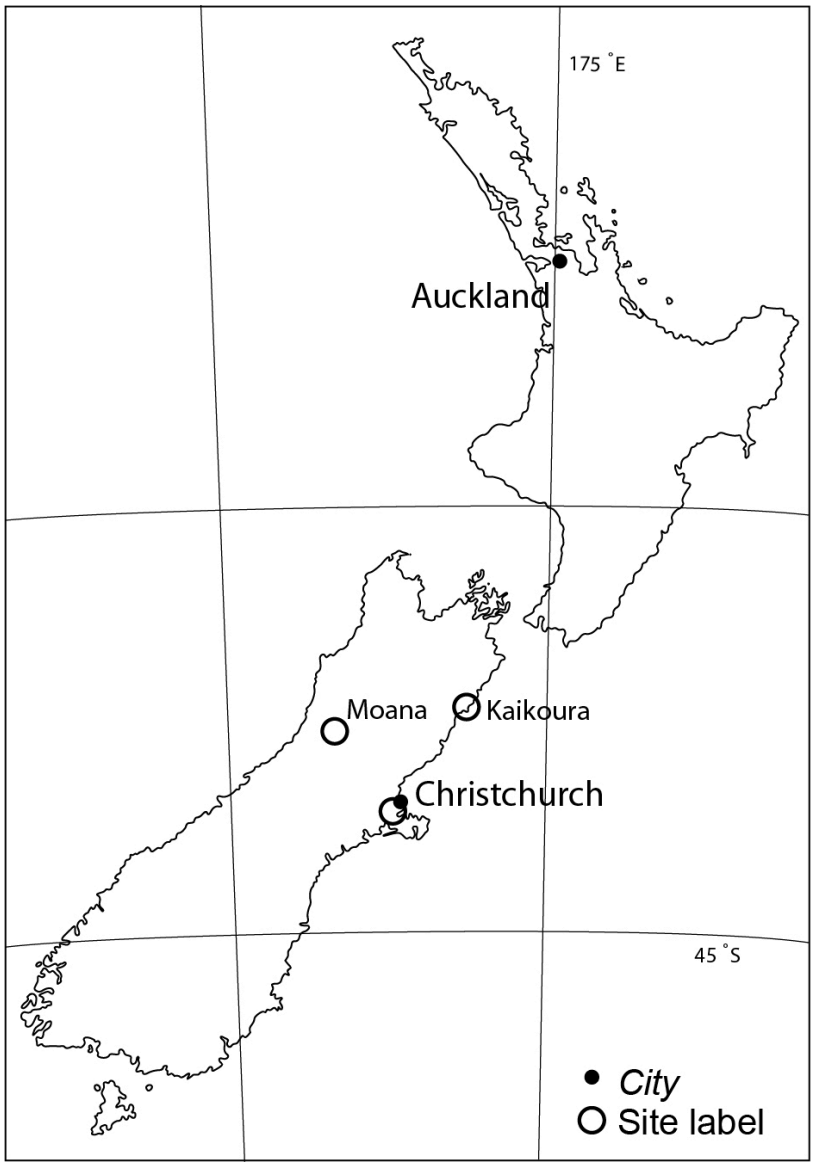

Figure 3.1: Locations of mist netting sites in the central South Island of New Zealand. Areas are situated between latitudes 42°S and 43°S. Hollow circles indicate the three study sites including, Christchurch, Canterbury, Kaikoura, north Canterbury, and Moana, West Coast.

Figure 3.2: Pox lesions present on all four digits of the right foot of an adult female silvereye (*Zosterops lateralis*) captured in Westmorland, Christchurch in the winter of 2010. Note that part of the second digit is missing and that tissue is showing signs of necrosis.

Figure 3.3: Two pox lesions, one 5.2 mm in diameter and the other 6.6mm, located on the second digit and the proximal end of the 8th, 9th and last primary feathers on the right wing of an adult silvereye (*Zosterops lateralis*). This individual was captured in the winter season at Westmorland, Christchurch, 2010.

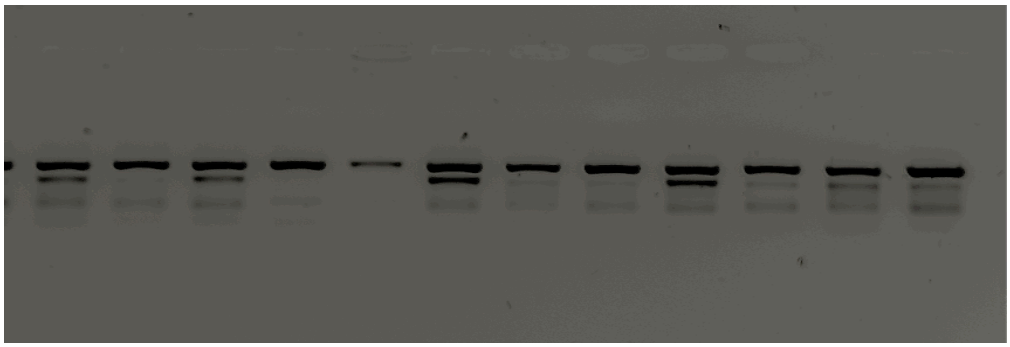

Figure 3.4: Part of a gel plate showing molecular sexing results for 12 silvereyes (*Zosterops lateralis*). PCR amplification was achieved using primers 2550F and 2178R. From left to right, sex was identified as F, M, F, M, M, F, M, M, F, F, F, F.

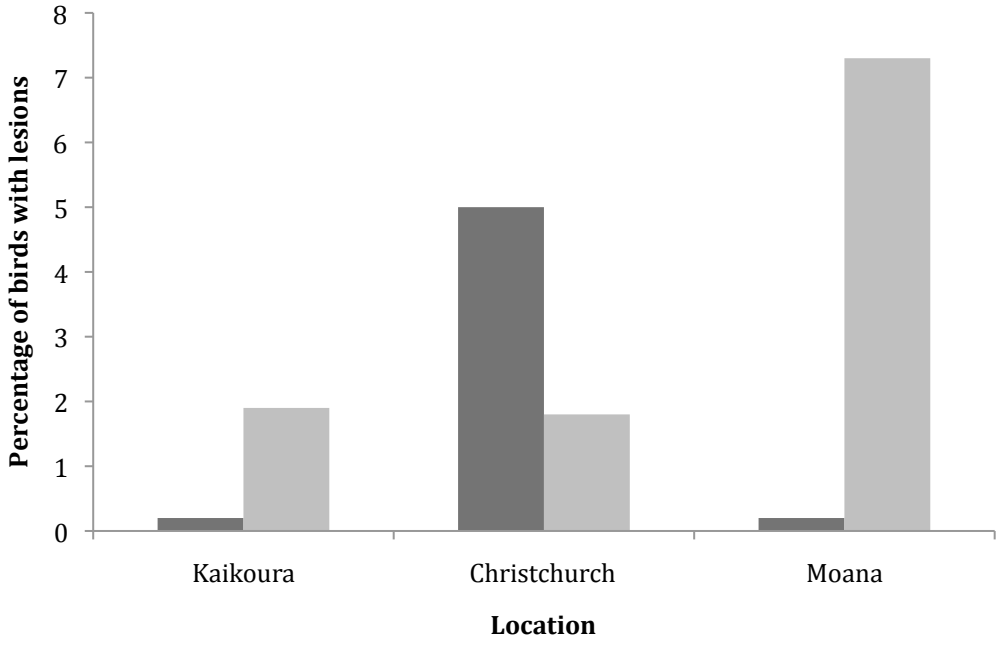

Figure 3.5: Seasonal changes in the percentage of silvereyes exhibiting symptoms of pox in each of the three survey locations. Dark shading represents 2010 and light 2011. Seasonal comparisons are as follows, Kaikoura (July 2010, vs. July 2011), Christchurch (June and July 2010, vs. February to April 2011), Moana (August and September 2010, vs. June 2011). All sexes and age groups combined in this analysis.

Figure 3.6: A female silvereye (*Zosterops lateralis*) with a suspected avian pox lesion on the corneal surface of the left eye. This individual was captured at Moana on the west coast of the South Island in June 2011.

Table 3.1: Measurements of the largest pox lesions detected on 9 individual silvereyes (*Zosterops lateralis*) captured in the South Island of New Zealand between June 2010 and July 2011. Severity is gauged as light if one lesion present, moderate for two, and heavy for three or more [methods follow those used by van Riper et al. (2002)].

Individual	Season	Sex	Severity of Infection	Number of lesions	Diameter of largest lesion (mm)	Area affected
Christchurch						
1	winter	F	moderate	2	6.6	Right wing
2	winter	F	heavy	4	3	Right foot, all toes
3	winter	U	moderate	2	Not measured	Left and right tarsus
4	summer	M	light	1	5	Base of right tarsus
Moana						
5	autumn/winter	F	light	1	1	Left foot, fourth digit
6	autumn/winter	M	light	1	5	Right leg, tibio-tarsal joint
7	autumn/winter	F	light	1	< 1	Left foot, first digit
8	autumn/winter	F	heavy	1	2	Cornea
Kaikoura						
9	winter	M	moderate	2	2	Right foot, first and second digits

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Chapter 4

Avian pox and its impact on New Zealand Silvereyes (*Zosterops lateralis*)

Abstract

Avian pox has been identified as an enzootic in some populations of silvereyes (*Zosterops lateralis*) in the South Island of New Zealand. An overall prevalence of 2.6% was estimated after completing surveys in three locations. Out of the 409 silvereyes surveyed for avian pox, nine adults and one juvenile were found to have pox-like lesions. A comparison of the morphology, mass, fat reserves and haematological profiles revealed a number of differences between healthy and infected individuals. Silvereyes with pox lesions had a slightly lower mass and less fat reserves than healthy individuals, while their white blood cell counts revealed lower heterophil to lymphocyte (H/L) ratios, heteropenia and eosinopenia, although none of these differences were statistically significant, likely as a result of the small sample of birds with avian pox. The potential negative impact on the health and survival of individuals infected with avian pox raises questions about the possible spread and impact of this disease on threatened endemic avian species in New Zealand.

Introduction

Much of our knowledge about avian pox has been gained through observing the disease in domestic poultry (e.g. Winterfield & Hitchner 1964; Gelenczei & Lasher 1968; Minbay & Kreier 1973; Tripathy & Cunningham 1984). Two main forms of the disease are recognised and are commonly referred to as dry pox and wet pox. The dry or cutaneous form of pox is caused by strains that infect the outer skin and lead to the development of smooth, nodular, wart-like skin lesions (van Riper & Forrester 2007). Lesions appear on non-feathered parts of the body, especially on the legs, feet and face, particularly the bill and eyes, including the eye surface (Figures 3.6 & 4.1). The wet or diphtheritic form of pox causes yellowish, cankerous growths on the mucous membranes of the upper respiratory tract and this type of infection is usually fatal (Tripathy & Reed 2008). Both forms can appear concurrently in an infected individual. Another form of avian pox that is less common, is a systemic form that has been seen in canaries, and such cases led to high mortality (Tripathy & Reed 2008). Although avian pox is highly infectious it is relatively slow to develop (van Riper & Forrester 2007). The main pathway of transmission is via the mouthparts of biting insects such as mosquitoes, midges, fleas and mites (van Riper & Forrester 2007; Smits et al. 2005). Skin abrasions also allow avipoxviruses to penetrate and invade epidermal tissue, indicating that transmission is possible via virus- harbouring dust particles (Tripathy 1993; van Riper et al. 2002).

In chickens, avian pox has been reported in some instances to infect just a few individuals while at other times, where little intervention has taken place, it has infected an entire flock (Tripathy & Reed 2008). Mortality is high in domestic chickens when the disease persists for longer than 3-4 weeks and where in the cutaneous form lesions form around the eyes or in the diphtheritic form in the respiratory tract (Tripathy & Reed 2008). While negative impacts such as weight loss, reduced egg laying capacity and breeding complications have been observed in chickens and turkeys, numerous deleterious conditions have also been documented in wild birds. Loss of vision (Karstad 1965), secondary bacterial infections leading to the loss of nails and digits (van Riper et al. 2002), predisposition to infection from malaria (Atkinson 2005) and comprised ability to successfully find mates (Kleindorfer & Dudaniec 2005) are some of the reported negative impacts of infection with avian pox.

With the recognition that avian pox is widespread and the publication of a number of studies documenting severe impacts on the health and survival of infected birds (e.g. Karstad 1965; van Riper & Forrester 2007; Tripathy & Reed 2008), concern over the disease is not limited to the poultry industry, but is also gaining the attention of conservation biologists concerned with its effects on wild bird species (e.g. Atkinson et al. 2005). However, few studies have measured the impact of avian pox on the condition of wild birds, although, a number have identified that avian pox has likely contributed to extinctions and dramatic declines of some endemic bird species on geographically isolated islands (e.g. van

Riper et al. 2002; Smits et al. 2005; Kleindorfer & Dudaniec 2006). For example, several endemic bird species in Hawaii have suffered negative impacts from avian pox that was previously absent and only introduced via contact with avian exotics and introduced mosquito vectors that transmit and spread the disease (van Riper et al. 2002). In Hawaii avian pox infections were shown to be significantly higher in native than introduced species, suggesting the native species were more vulnerable (Jenkins et al. 1989; van Riper & Scott 2001; van Riper et al 2002). The work in Hawaii lends strong support to the hypothesis that avian pox can be a limiting factor on bird populations. The studies also highlight the danger of introducing avian pox to bird populations that are naïve to virulent strains of this disease and raise questions about the vulnerability of other bird species in different parts of the world where they may be faced with similar scenarios.

New Zealand shares similarities with Hawaii in terms of its geographical isolation, removal of natural habitat and the introduction of exotic bird species. Furthermore, many of New Zealand's endemic birds have experienced severe genetic bottlenecks, a phenomenon linked with increased vulnerability to disease (Briskie & Mackintosh 2004; Hale & Briskie 2007; Heber & Briskie 2010). Population bottlenecks in New Zealand have been further exacerbated through past hunting and the introduction of mammalian predators, especially rats and mustelids (e.g. Holdaway 1999). As of 2012, the New Zealand Department of Conservation (DOC) listed 24 bird species as nationally critical, 15

species as endangered, and another 38 species as vulnerable. It is not known whether avian pox is a significant cause in the decline of any of these species but similarities between Hawaii and New Zealand add weight to the importance of improving our knowledge about the epizootiolgy and impact of avian pox on native birds in this country. Avian pox has been reported previously in New Zealand. It was observed in the New Zealand pipit (*Anthus novaeseelandiae*) in the 1950's (Westerkov 1953) and in silvereyes (*Zosterops lateralis*) in the 1970's (Austin et al. 1973). More recently it has been identified in as many as 15 different species (Ha et al. 2011). A recent investigation on a group of inbred New Zealand robins (*Petroica australis*) in 2010 on islands in the Marlborough Sounds led to the discovery that some individuals had extensive pox lesions (S. Heber, *pers. comm*.). Despite evidence that the disease is present in avian populations in New Zealand there still remains a gap in our understanding about the impact of the disease on individual birds or populations and little is known about the number of strains, virulence or method of transmission.

In this study I investigated the impact of avian pox on the New Zealand silvereye (*Zosterops lateralis*) and used body condition and haematological profiles to assess differences between healthy and infected individuals. The silvereye is a small passerine belonging to the white-eye family (Zosteropidae) and is a recent natural colonizer of New Zealand, originating from Tasmania (Estoup & Clegg 2003). Like other New Zealand species it has also undergone a population bottleneck with

silvereyes establishing themselves as a series of founder groups in the mid nineteenth century (Falla 1953). Estoup & Clegg (2003), conclude that the bottleneck event for silvereyes was of low intensity, nevertheless, any loss of genetic variation could be a factor influencing the impact of disease on silvereye populations making them a suitable group for studying the effects of avian pox on a single species.

Methods

To determine the effects of avian pox on silvereyes, I captured and surveyed birds at three locations in the central South Island of New Zealand (figure 3.1). Survey work was carried out over a period of two years in different seasons (June- August, 2010 and February-July, 2011). Locations included: (1) Westmorland, a hill suburb in southwest Christchurch, Canterbury, (43°34'59 S, 172°36' E; elevation 90 m), (2) Kowhai Bush, Kaikoura, (42°22'34 S, 173°36'57 E; elevation 72 m) and (3) Moana, Lake Brunner, West Coast, (42°34'29 S, 171°28'39 E; elevation 112 m). The distance range for the three locations is 140 km to 185 km and therefore allows comparison between different populations as well as locations with different climates (see chapter 2).

Silvereyes were captured using a single 6m x 2.5m mist-net placed close to trees where birds were lured using a 50:50 mix of beef dripping and sugar smeared over suspended pinecones. Nets were placed at ground level and observers were situated no further than 25 m away allowing

nets to be checked regularly to ensure that ensnared birds could be released immediately for assessment.

Once captured, birds were placed in a cloth bag. Mass was measured to the nearest 0.1 g (using a Jever digital scale) and then birds were banded using aluminium A size bands, issued by the New Zealand Government's Department of Conservation. Fat reserves were estimated using a scoring system on a 0-5 continuum scale using methods described by Krementz & Pendleton (1992). Superficial deposits of fat in birds are primarily stored under a thin layer of skin below the chin. By blowing gently on the contour feathers it is possible to observe the relative amount of any fat stored and to obtain a score. Measurements of tarsus, bill and non-flattened wing chord length, were also recorded. I then obtained a blood sample from the brachial vein using a 0.33 X 13mm needle. No more than 60 μL blood was collected with a heparinised capillary tube. Using this sample I created a blood smear on a frosted microscope slide with the use of a second slide as a spreader. Slides were left to air dry and later fixed in absolute methanol for 5 minutes. Remaining blood was immediately placed into a small microtube containing 1.5 ml solution of Queen's lysis buffer (Seutin 1991) for later DNA analyses to sex birds. To facilitate the reading of smears with a light microscope, all slides were stained using May-Grunwald Giemsa (Lucas & Jamroz 1961). In cases where captured silvereyes presented with lesions, individuals were measured, photographed and if lesions were large enough, a scraping was taken with a small scalpel for future PCR work in order to confirm and identify

strains of avipoxvirus. Growths were identified as lesions if they were greater than 0.5 mm in diameter and had the smooth, pink, nodular appearance described in van Riper & Forrester (2007). Infections were rated as light, moderate or heavy; a 'light' infection was defined if only one lesion could be seen, 'moderate' if there were two lesions and heavy when three or more lesions were noted or lesions were found on the head (van Riper et al. 2002).

Haematological analyses, morphological data and fat scores were used to make comparisons between non-infected and infected silvereyes. Estimation of body condition was primarily based on measurement of body mass and fat score data. These parameters were treated separately to allow the direct comparisons between groups as debate still exists about the accuracy and consistency of ratios when they are used to produce meaningful indices for body condition (Green 2001). Mass and fat deposits in passerine species are known to fluctuate throughout seasons and throughout the day (e.g. Baldwin & Kendeigh 1938; Dawson et al. 1983; Koivula et al. 1995; Cresswell 1998; Rintamaki et al. 2003). To reduce variation created by diurnal fluctuations in mass and fat stores, I analysed data collected in the morning (before 12 noon) separately from the data collected in the afternoon.

The collection and analyses of blood is acknowledged as a useful method of gaining information about bird health provided that consistent methods are used (Dein 1986; Fudge 2000; Davis 2008), however,

limited research has been carried out on WBC differentials in wild birds and little information exists to provide reference values in haematology for most avian species (Davis 2008). There are no reference values for silvereyes. Difficulties exist with electronic counts of white blood cells (WBC) due to interference from nucleated erythrocytes and thrombocytes and so this procedure is usually done manually (Campbell 1994). I conducted manual counts of WBC's and calculated differentials for the various types of leucocytes in order to detect any significant differences (tables 4.3A & 4.3B). Elevated levels of WBC's (leucocytosis) can be an indication of immunological response to parasites, bacterial or viral infections (Fudge 2000). Comparing the ratios of various types of leucocytes is a method used to detect increased levels stress; for example, elevated numbers of lymphocytes (lymphocytosis) has been correlated with the onset of disease (Fudge 2000). Heterophils (the equivalent of neutrophils in mammalian species) can also increase in number during periods of physiological or social stress in birds (Gross & Siegel 1983; Fudge 2000). Investigating immunological response to stressors through the comparison of the number of heterophils to lymphocytes (H:L ratio) has been used as an assessment tool for gauging stress in domestic poultry and is regarded as a more accurate form of measuring stress (e.g. Gross & Siegal; Maxwell 1993; Lethey et al. 2000; Mashaly et al. 2004). This method was used in my study and I obtained these values by dividing the number of heterophils by the number of lymphocytes. Increases or decreases in the number of other types of leucocytes (monocytes, eosinophils and basophils) are also indicators of

immunological responses to disease (Fudge 2000) and these cell types were counted. Thrombocytes (small nucleated cells that assist with blood coagulation) were also counted because these cells are known to react to the presence of pathogens (Fudge 2000).

While leukocyte profiles have been used to gauge stress in vertebrates including avian species, leukocyte morphologies can also be used as indicators of physiological or disease-related stress (Campbell 1994, Fudge 2000). The presence of immature heterophils is considered abnormal and likewise, so is any toxic change in mature heterophils such as increased cytoplasmic basophilia or vacuolation and degranulation or degeneration of the nucleus (Campbell 1994). An increase in size or cytoplasmic basophilia is an indication of reactivity in lymphocytes (Campbell 1994). If large numbers of monocytes develop vacuolated cytoplasms, this is also considered to be abnormal cytology.

Slides were examined under 400 X magnification to obtain an estimated white blood cell count. An across, up, across, down method was used for selecting and viewing respective fields in order to avoid repeat counts of cells. After counting leucocytes in ten consecutive fields, the average was determined (total/10) then doubled to obtain a total estimated WBC count (x 10^9/l). Thrombocytes were counted and averaged using the same fields where the total was then divided by 10 to obtain the average. To differentiate between the various leucocytes (heterophils, eosinophils, lymphocytes, monocytes and basophils), slides were viewed under 1000

X magnification. Cell types were then calculated as proportions (cell type/total number of white cells counted). This was followed by another calculation of absolute proportions (x 10^9/l) where the score for each cell type was derived by dividing the proportion by the total estimated WBC count (Campbell 2007). Comparisons of blood cell composition were made using samples from all seasons and locations with most samples derived from the second year due to damaged slides in the first season.

Silvereyes are difficult to sex reliably while in the hand; therefore molecular sexing was used as a means of identification (figure 4.4). DNA was extracted from blood using a SIGMA, XNAB2 kit. Blood volumes of 10 μL were added to an equal amount of lysis then left to stand for five minutes, before adding 90 μL of blood neutralization solution. Samples were covered with foil and refrigerated at 4°C. Polymerase chain reactions (PCR) were performed using a mix ratio of 5 μL molecular water, 1 μL of primer (avian sex primers 2550F and 2718R), and 10 μL of the enzyme Kapa A, blood mix B (KapaBiosystems KM7005). Samples were run on an Eppendorf thermal cycler with the same heating and cooling profile as recommended by Fridolfsson & Ellegreen (1999). PCR product separation was achieved with a 1.5% agarose gel mix and SYBR safe DNA gel stain was used for visualisation. All electrophoresis was carried out for a period of 30 minutes at a setting of 120 volts.

Appropriate permits were obtained from the New Zealand Department of Conservation and University of Canterbury's Animal Ethics Committee for capture, handling, banding and taking blood samples.

Results

Out of the total capture of 409 silvereyes, 10 cases of the cutaneous form of avian pox were detected using visual symptoms to identify lesions (see methods). Of the 10 cases, 9 of these were adults with one juvenile found in Christchurch by a local resident. Five cases were classified as light infections, three as moderate and two as heavy. Sex was determined in 7 out of the 9 infected adult birds and 5 of these were females. Lesions ranged from 1mm to 6.6 mm in diameter and were mostly found on the legs and feet. In one silvereye a lesion was found on the cornea and in another individual two lesions were found on one wing. The two heavy infections were observed in females (table 3.1).

Morphology, Mass and Fat Scores

Morphological comparisons of silvereyes with and without avian pox are summarized in table 4.1. Sample size was too small to analyse each sex and geographic location separately, but there was no evidence to suggest that pox had any effect on the physical size of individuals when the data from all sites was combined (table 4.1). However, it is interesting to note that comparisons of adult infected birds with non-infected adults reveal little difference in terms of mean mass (0.1 g in favour of the non infected

group). The five infected females (12.4 g) were on average 0.69 g (4.6%) lighter than females without lesions (13.04 g), although this difference was not significant (t-test: t = 1.9, df = 142 , P = 0.23). Three of the infected females were identified in Moana and were 3% lighter than the rest of the females (n=29) captured there in the same year. One of the three individuals with lesions in Christchurch in the winter of 2010 and the only one with a measurement for mass can be seen in figure 3.2. This individual weighed 11.3 grams and had a fat score of zero. When compared with the rest of the females in the same location for 2010, its mass was 14.8% less than average and its fat score was low when compared to the group mean of 2.5. Differences in fat scores between healthy silvereyes and those with pox are not significant (Mann-Whitney U-test: U = 822.5, P = 0.98, n = 275 and 6).

Haematology

I was able to carry out WBC counts on 46 blood smears from birds without pox lesions and the calculated reference values can be seen in table 4.2B. The most common leucocytes in this non-infected group were lymphocytes with a mean absolute proportion of 14.9 x 10^9/l (range 5.8-44.3). The next most frequent cell types were heterophils, 2.22 x 10^9/l (range 0-16.3) and monocytes, 2.45 x 10^9/l (range 0.7-8.01).

Comparisons of WBC differentials were made between healthy males and infected silvereyes of both sexes but statistical analysis was limited due to the low number of blood samples obtained from infected birds (n = 5). When comparisons were made between all non-infected and infected

birds across all seasons, the biggest differences were seen in absolute proportions of heterophils, eusinophils, as well as in H/L ratios, but none of these were statistically significant (table 4.2C). The overall estimated WBC count mean was lower in the infected group (17.65 (n = 5, range 13.4-21.4) than the non-infected group 21.22 (n = 45), range 9.4-49.8) but this too was non- significant. I was unable to find any evidence of the blood parasites *Haemoproteus* or *Plasmodium*.

For the non-infected silvereyes there were some differences between males and females. The mean values for the H/L ratio and the absolute proportion of heterophils in males (H/L 0.11, n=19, range 0.01-0.87), were less than half of the values calculated for females (H/L 0.23, n=21, range 0.01-0.84). Comparing the means for absolute proportion of lymphocytes in this same group of non-infected males and females revealed that males (17.7, n=19, range 6.88-44.37) had a significantly higher proportion of this cell type than females (13.68, n=21, range 5.8-28.3). Average total thrombocyte counts were slightly lower in healthy females than in all other groups including infected birds.

The largest sample of blood smears was obtained from Moana in July 2011. Although small samples make statistical analysis problematic, I found that almost all of the mean values for WBC differentials for infected females were similar to non-infected females. The H/L ratio mean was only slightly higher in the infected group of females compared to healthy females (0.074 versus 0.06) while the eosinophil mean absolute

proportion was lower in infected females than healthy females (0.54 versus 1.43). The most obvious difference was seen in the mean number of thrombocytes where healthy females had an average of 4.1 cells per field (range 13-95) while infected females averaged 9.2 (range 53-168). One infected female with an eye lesion had a higher absolute proportion of monocytes (5.13) compared to the non-infected female median of 1.84 (n = 20). While inside the third quartile, such a result could be regarded as monocytosis. One female not presenting with lesions had an absolute monocyte proportion of 8.01, suggesting evidence of leucocytosis, specifically, heterophilia, eosinophilia and lymphocytosis. This may indicate an infection and lends more weight to monocytosis in the female with the eye lesion. Comparisons between non-infected males and non–infected females within the 2011 Moana group revealed differences in mean values for white blood cell composition. The estimated WBC average count was lower (20.9 vs 28.75), eosinophils higher (1.43 vs 0.8) and lymphocytes lower (15.1 vs 24.2) in females than males respectively.

As well as leukocyte profiles, comparisons were made between morphologies of blood cells in healthy silvereyes compared to those with pox lesions. An adult male silvereye captured in Moana with a large 5mm diameter lesion on one of its legs (figures 4.3B, 4.3C & 4.3E) showed a marked increase in the number of immature erythrocytes (rubricytes) indicating regenerative anemia (Dein 1986)(figure 4.4C). This infected adult male's lymphocytes were fewer in number 7.11 compared to the non-infected males (mean = 24.12 for Moana (n=4) and 17.79 in all

analysed males (n=19). Monocytosis was observed with an absolute count of 5.12 compared to 2.84 (Moana bird only) or 2.17 (all analysed males range 0.85-4.8). Many of the monocytes and lymphocytes had vacuolised cytoplasms or showed signs of blebbing; both of these phenomena are regarded as likely reactive changes to viral infection (Maxwell 1993). One other unusual feature seen in one of the infected females was an increased number of immature heterophils, rarely seen in other smears and their presence is considered to be abnormal (Campbell 1994).

Discussion

Studies indicate that avian pox is present in a wide a range of bird species but our understanding of the disease's impact on wild bird species is limited. More recent investigations on the effects of avian pox on isolated bird species show that it has had the most devastating effect on island endemics. Speculation that avian pox may indeed be a significant problem for silvereyes because of inbreeding depression is not supported by my findings. My investigation revealed a relatively low prevalence of the disease in silvereyes for three areas in the South Island of New Zealand (< 3.7%) over a two-year period. Such a low prevalence made it difficult to use statistical evidence to gauge the comparative impact of avian pox on individuals due to the reduced the probability of capturing infected silvereyes. Despite the small number of birds presenting with pox lesions,

I was still able to use morphological information and haematological profiles as indicators of the possible effects of dry pox on condition.

Impact of lesions on limbs, mass and fat reserves

Pox lesions could compromise a bird's ability to fend for itself, particularly if lesions are located around the head and eyes, and thereby interfering with vision. Such a phenomenon was observed in captive Impiyan pheasants (*Lophophorus impejanus*) where eye lesions were responsible for substantial losses (Karstad 1965). Indeed if the ability to forage is impeded by the presence of lesions it would be logical to predict that infected birds should have a lower body mass and lower fat reserves than their healthier counterparts. I captured one silvereye with an eye lesion located on the cornea of its left eye, however, this particular female had a high fat score and its mass was above the mean for the other females captured in the same area and season. The largest difference in mass and fat score was observed in a silvereye female captured in Christchurch with several lesions on its toes (figure 2.2). This individual was 2.3 g lighter than the average mass of other females caught at that time in the same area and had a fat score of zero. A zero score is unusual for an afternoon reading (mean 2.45) and with this particular female group (n=11) only one other female had a fat score rating of zero but had a well above average mass of 14.2g. My results do indicate a possible reduction in mass and fat reserves due to impediment from infection with avian pox, but a larger sample is needed to confirm this.

A number of studies have reported diurnal changes in body mass in small passerine species, where mass progressively increases towards the evening (e.g. Partin 1933; Owen 1954; Helms and Drury 1960; Koivula 1995) and a variety of hypotheses have been put forward to explain this. The purpose of recording the time of measurement when obtaining data for mass in my study was to take into account diurnal fluctuation in mass to improve accuracy when making comparisons between groups. It is recommended that similar methods should be used to take into account change in mass where comparisons require mass is to be used as a dependent variable.

While lower mass and depleted fat reserves may be a direct or indirect consequence of avian pox infection, the direct effects of lesions on the limbs of infected silvereyes is pronounced (see summary of lesion numbers and sizes discussed in chapter 3). A single juvenile silvereye in Christchurch had a 5mm lesion at the base of its right leg that had caused a deformity where the entire leg had rotated 45° to the right (figure 4.2A). In two of the infected adults where more than one lesion was present on the feet, some lesions had previously burst and subsequent bacterial infections had led to the loss or partial loss of digits. In summary the cutaneous form in three out of the nine cases we observed had a major effect on the condition of the limbs. In the previous chapter I raised the issue of a possible sampling bias where individuals severely debilitated by the disease are less likely to be caught. In cases where the lethal diphtheritic form has taken hold these individuals are unlikely to

be caught at all. Given that three of the nine cases were severe enough to cause deformities to the limbs, the possibility remains that there are many more heavy cases of avian pox in the silvereye population which remain undetected as these birds struggle to survive for very long.

White blood cell counts and cell morphology

Hematological information gained from a complete blood count provides useful information about the condition of a bird (Dein 1986). A complete blood count makes it possible to detect abnormalities such as anemia, leucocytosis, lymphocytosis, abnormal erythrocyte morphology and many other types of immunological responses that may arise from stress for a vast array of reasons (Fudge 2000). Analysis of the 5 blood smears obtained from infected individuals revealed a number of differences when compared to smears from healthy birds. While similarities were evident when comparing white blood cell differentials of infected with non-infected females in Moana, anomalies were still present in some infected individuals in terms of WBC profiles, thrombocyte counts as well as WBC and erythrocyte morphologies (figures 4.3 & 4.4). Indeed, these aspects were very different in the case of the one infected adult male captured in this location. When presented with an individual sick bird, veterinary clinicians often use haematological information to make comparisons between the patient and what is known about healthy birds in terms of WBC profiles, red blood cell counts and cell morphology. One of the most dramatic differences seen in all five of these infected individuals was the reduced H/L ratio (table 4.2A). The heterophil to

lymphocyte ratio is regarded as one of the more accurate measures for indicting reaction to stress (e.g. Gross & Siegal 1983; Davison et al 1983; Maxwell 1993; Lethey et al. 2000; Mashaly et al. 2004). In the early 1980s Gross and Siegal used corticosteroids to induce stress in chickens (*Gallus gallus*) and measured the changes and responses in blood tissue. One of the observed responses was a reduction in the numbers of lymphocytes and an increase in heterophils. Subsequent studies also correlated this response in chickens when they were subjected to other forms of stress. As lymphocyte numbers decrease and heterophils increase relative to each other the H/L value increases. When comparisons were made between the mean value of birds with lesions and those without in my study, I did not observe any increase in the H/L value, in fact, the H/L ratio was considerably lower in each individual case. A review paper by Maxwell (1993) concludes that while H/L ratios provide a more reliable indication of stress levels in mild to moderate cases of stress, this is not true in cases where the level of stress is extreme and heteropenia and basophilia is common. Very low H/L ratios in all five infected samples suggest that these birds may indeed have been under severe stress, perhaps as a result of infection with avian pox.

Leucocytosis is commonly caused by an increase in heterophils in the peripheral blood and is considered to be a result of stress or inflammation (Fudge 2000). I observed little change in the overall WBC count and in all infection cases the number of heterophils was considerably lower than the mean heterophil value for non-infected male

or female groups. I also observed a relatively low heterophil count in healthy silvereyes when compared to other species. Some species do have a relatively low heterophil-to-lymphocyte ratio, for example canaries and some Amazon parrots (Fudge 2000). My findings suggest that heterophils circulate in lower numbers in silvereyes.

Heteropenia is regarded as uncommon and could be the result of a reduction in the presence of heterophils due to damage when creating a smear. The appearance of many smudge cells (ruptured cells on a smear) may be an indication that many white cells have been damaged and this can lead clinicians to falsely reporting lymphocytosis (Fudge 2000). Blood smears obtained in the first season were not used for analyses for this very reason. A significant number of the smears had large numbers of smudge cells present. The smears obtained in 2011 were of better quality and smears were not used for analysis if smudge cells were a problem. Selecting smears with low numbers of smudge cells was an important aspect of increasing the accuracy of reading blood profiles. The technique used to create smears is important because white cells are delicate and easily damaged. I recommend that high quality slides should be used as spreaders whenever two slides are used to create a smear.

Monocytes vary significantly in size and this can cause difficulties in differentiating them from lymphocytes. There is a tendency for these cells to be deposited on the periphery of smears and I also observed this phenomenon. Monocytosis is often seen when inflammation is present

and when bacterial infection has developed. I observed this condition in two of the five infected cases, however, I was unable to observe a patterned response. Eosinophil numbers, however, were lower in every case for the infected samples. Eosinopenia is associated with the effects of corticosteroids and stress (Monks & Forbes 2007). A significant drop in eosinophils was observed in Harris's hawks (*Parabuteo unicinctus*) when subjected to transportation (Parga et al. 2001) and also in wedge-tailed shearwater chicks (*Puffinus pacificus*) during a stranding episode in Hawaii (Work & Rameyer 1999). Turkeys inoculated with MC29 virus (avian myelocytomatosis) succumbed to infection and significant changes were observed in WBC profiles including lymphocytosis, heteropenia and eosinopenia (Nagy et al. 1981). Eosinopenia has been induced in rabbits where acute inflammation post infection with trichinosis led to a rapid decline in circulating eosinophils. All five infected silvereyes showed signs of eosinopenia with deviations from -23.3 % to a -90% from mean values according to sex and suggests that these individuals may be stressed as a result of infection with avian pox.

Small, nucleated cells known as thrombocytes are considered to be important cells for the coagulation of blood in birds, in contrast a function that involves fragmented tissue in the form of platelets in mammals. When smears are examined these cells are normally reported for their frequency and also morphology as blebbing or enlargement of the cytoplasm can indicate a reaction to infection (Fudge 2000). Reduced thrombocyte cells (Thrombocytopenia) were observed in chickens

infected with Newcastle disease virus (Calderon et al. 2005) and Asian H5N1 influenza (Swayne 2007; Suzuki et al. 2009). A reduced frequency of this cell type in four of the five blood profiles obtained from infected silvereyes may well be a similar tissue response to that seen in chickens when challenged with viral infection. I observed a tendency for these cells to occur in clumps and that the variation in counts between general individuals was very high (range 13-305). A number of different factors may well be involved in their number and distribution including smear technique (Fudge 2000).

While some common patterns were identified in blood profiles for the small sample of silvereyes infected with pox, further work is needed to develop specific references that are indicative of the stress caused by viral infection with avian pox. Changes in cell morphology were clearly observed in some cases (figures 4.3 & 4.4), however, a larger sample size is needed to establish typical changes in cell morphology in the peripheral blood of silvereyes as a response to infection with avian pox .

Implications for conservation in New Zealand

Little is understood about the number, type or virulence of avian pox strains in New Zealand. We don't have information about whether certain species act as specific hosts or whether some strains can adapt to a range of hosts. The risk that avian pox poses to other avian species while classified as an enzootic disease in silvereyes remains unknown. Silvereyes are widespread, abundant and migratory and potentially could

act as a large reservoir for the spread of avian pox. The likelihood of existing strains or the cross over of a new strain from introduced birds to the silvereye population and whether this could cause a problem for New Zealand avian endemic species is an issue that warrants further investigation.

Where endangered species have been through bottlenecks in New Zealand there is evidence that these populations are vulnerable to diseases like avian pox, for example saddlebacks (*Philesturnus carunculatus*) (Hale & Briskie 2007), Black robins (*Petroica traversi*) in the Chatham Islands (Tisdell & Merten 1988: M. Massaro, pers. comm. 2012) and South Island robins (*Petroica australis*) (Heber et al. 2013). The Hawaiian avifauna has experienced extinctions and devastating population depletion in many species (van Riper & Scott 2001). Severe declines in populations of rare endemic birds are also documented in the Galapagos Islands (Kleindorfer & Dudaniec 2006) and the Canary Islands (Smits et al. 2005). A common thread in all of these studies is that avian pox has been identified as a major contributing factor to the demise of bird populations existing on geographically isolated islands. With the potential of diphtheritic pox to kill birds outright and evidence that cutaneous forms of the disease can cause physical deformities to limbs, impair vision or increase physiological stress, this is a disease that needs to be taken seriously in New Zealand where so many threatened species reside.

Figure 4.1: This pox lesion (2mm in diameter) was observed on the second digit of the right foot of a male silvereye (*Zosterops lateralis*) at Kowhai Bush in Kaikoura in the winter 2011.

Figure 4.2A: A juvenile male silvereye (Zosterops lateralis) presenting with a large lesion on the right leg. This individual was found in the suburb of Ilam by a Christchurch resident in February 2011.

Figure 4.2B: This large lesion seen on the same juvenile described in figure 2A has led to deformed growth of the toes.

Table 4.1: Comparison of physical measurements of silvereyes with and without pox lesions. Bill length measurements are from the nares to tip and wing chords were not flattened.

Trait	Non-Pox (N)	Pox (N)	t	P
Body mass (g)	13.11 ± 0.07 (272)	13.03 ± 0.56 (6)	0.15	0.88
Tarsus (mm)	20.62 ± 0.05 (277)	20.41 ± 0.36 (7)	0.66	0.51
Wing (mm)	61.42 ± 0.11 (278)	60.43 ± 0.69 (7)	1.4	0.16
Nares (mm)	7.19 ± 0.03 (278)	7.01 ± 0.19 (7)	0.98	0.33

Table 4.2A: Estimated white blood cell counts (number of leucocytes $X10^9$/l) and heterophil to lymphocyte ratios when grouped by sex and infection status.

Group	n	Estimated WBC	Range	H/L Ratio	Range
Non-pox Female	20	21.36	9.6-46	0.23	0.01-0.84
Non-pox Male	20	22.97	9.4-49.8	0.11	0-0.87
All non-pox birds	46	21.22	9.4-49.8	0.17	0-0.87
Birds with pox	5	17.65	13.4-21.4	0.068	0.01-0.17

Table 4.2B: Mean absolute white cell proportions where the number of specific leucocytes was divided by the total white cells counted and expressed as a fraction. This was then multiplied by total estimated white blood cell count and presented as a proportion $X10^9$/l (see methods)].

Group	Non-pox Female	Non-pox Male	All non-pox birds	Birds with pox
n	20	20	46	5
Heterophils (Range)	3.13 (0.14-16.03)	1.42 (0-6.04)	2.22 (0-16.03)	0.98 (0.17-2.62)
Lymphocytes (Range)	13.68 (5.8-28.3)	17.7 (6.88-44.37)	14.96 (5.8-44.37)	13.4 (7.11-17.3)
Eosinophils (Range)	1.78 (0.34-12.35)	1.53 (0.09-6.03)	1.5 (0-12.35)	0.77 (0.17-1.15)
Monocytes (Range)	2.67 (0.7-8.01)	2.17 (0.85-2.68)	2.45 (0.7-4.28)	2.43 (0.89-5.13)
Basophils (Range)	0.038 (0-0.23)	0.021 (0-0.24)	0.02 (0-0.24)	0 (0)
Thrombocytes (Range)	7.29 (1.4-28.5)	8.87 (2.1-30.5)	8.27 (1.4-30.5)	8.42 (5.3-16.8)

Table 4.2C: A statistical comparison of haematological values between healthy silvereyes and those with avian pox. (WBC = white blood cell count, H = heterophils, E = eosinophil L = lymphocytes, M = monocytes, B = basophils and H:L = heterophil to lymphocyte ratio)

Trait	Non-pox	Pox	t	P
Estimated WBC count	22.1 ± 1.6	17.8 ± 1.3	0.75	0.46
Abs prop H	2.3 ± 0.47	0.89 ± 0.44	1.45	0.15
Abs prop E	1.66 ± 0.34	0.69 ± 0.2	1.46	0.15
Abs prop L	15.7 ± 1.3	13.3 ± 1.7	0.5	0.62
Abs prop M	2.43 ± 0.25	2.97 ± 0.9	- 0.62	0.54
Abs prop B	0.03 ± 0.011	0	0.98	0.33
H/L ratio	0.18 ± 0.03	0.07 ± 0.03	1.15	0.26

Table 4.3: Haematological comparisons for silvereyes presenting with lesions in five cases where smears were successfully obtained and white blood cell profiles established with the addition of thrombocytes. (E-WBC = estimated white blood cell count, H = heterophils, L = lymphocytes, H:L = heterophil to lymphocyte ratio, E = eosinophil, M = monocytes and T = thrombocytes). Figures show the percentage increase or decrease from the mean score according to gender.

Infected Individual and severity of infection	Sex	E WBC	H	L	H:L	E	M	T
1. Light	M	-25	-52.9	-22	-63.6	-23.3	-43.1	-33
2. Light	M	-41.5	-65	-59.9	-54	-56	+96.9	-36.9
3. Light	F	0	-16.1	+12.5	-26.1	-35.3	+7.1	+130
4. Light	F	-22.7	-94.5	+27.2	-95.6	-90	-57.6	-27.3
5. Heavy	F	-22.7	-88.7	-8.1	-82.6	-79.4	+144	-24.5

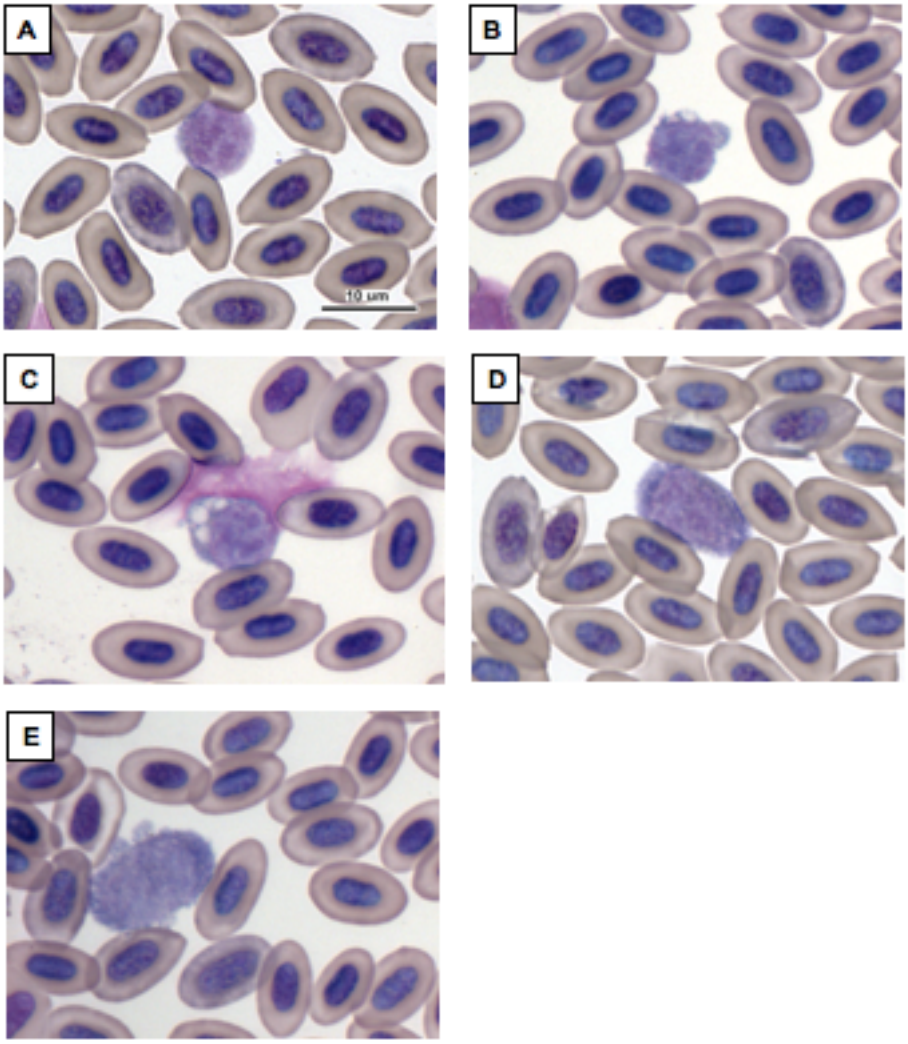

Figure 4.3: A lymphocyte from the peripheral blood of a healthy male silvereye (A). A lymphocyte with blebbing and vacuolization of the cytoplasm from the peripheral blood of a male silvereye infected with avian pox (single large pox lesion on one limb) (B). A lymphocyte showing cytoplasmic vacuolization from the same infected male silvereye described above (C). A monocyte from the peripheral blood of a healthy male silvereye. (D). Blebbing in the monocyte of a silvereye identified with avian pox. (E). (The scale drawn in fig. 4A. represents 10 microns. All blood films have been stained with May-Grunwald Giesma).

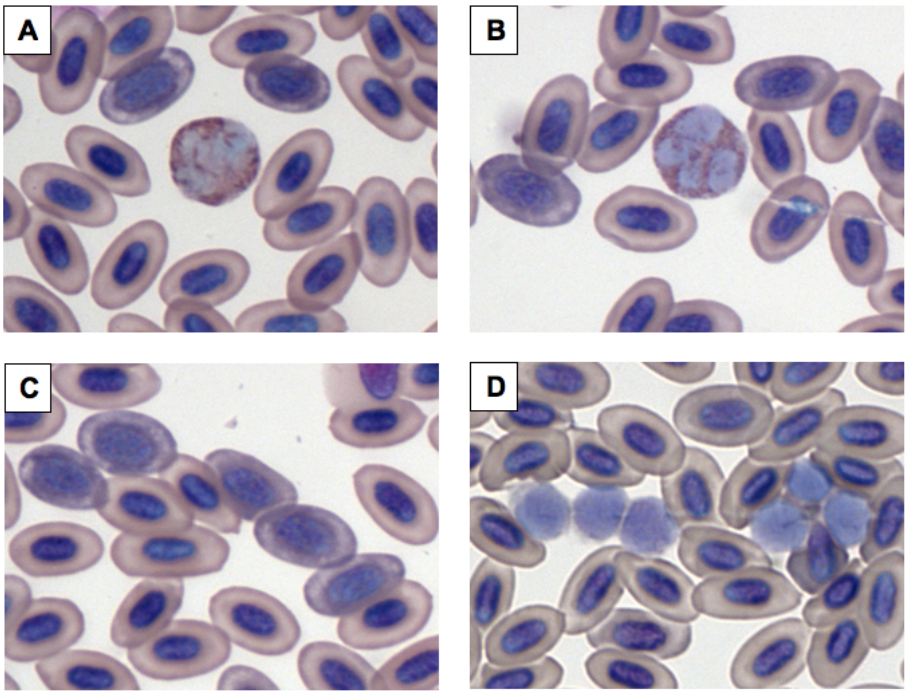

Figure 4.4: A selection of blood cells seen in the peripheral blood of a male silvereye identified with avian pox. An example of a heterophil (A). An eosinophil (B). An example of young erythrocytes seen in large numbers in all 10 fields used for analyses (C). An example of clumped thrombocytes (D). All films stained with May-Grunwald Giemsa.

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Chapter 5

General discussion

In this thesis I investigated the prevalence and impact of avian pox on different South Island populations of New Zealand silvereyes (*Zosterops lateralis*), a common, self introduced species from Tasmania. I also conducted a broader investigation about the prevalence of avian pox through a survey of several different passerine species in a single location in the South Island. Comparisons were made between the incidence of avian pox in this study and similar bird species overseas. A key aim in this investigation was to provide more information about the possible threat posed by avian pox to birds in New Zealand through a better understanding of the disease in silvereyes.

At the culmination of my field season in 2012 (chapter 2), 492 native and introduced birds were captured in Kowhai Bush, Kaikoura. The birds were screened for avian pox and I found that the incidence for this disease was low. Surveys concentrated on the detection of avian pox in silvereyes in three locations of the South Island over a period of three years also revealed a low incidence of this disease. To obtain references about the prevalence of avian pox in passerine species and to provide an overall perspective I analysed worldwide literature where occurrences of avian pox were quantified. Furthermore, I made comparisons between groups of species according to degrees of residence in order to find any differences in the prevalence of the disease according to degrees of

residency (chapter 3). I found that avian pox was significantly more prevalent in endemic birds living on geographically isolated islands. Studies focusing on isolated endemic species correlated epizootics of avian pox both with the introduction of avian stock and high densities of biting insects. Furthermore these studies identified that avian pox and avian malaria were leading causes of severe population declines in avian endemics, especially in Hawaii the Galapagos Islands and the Canary Islands (e.g. Jenkins et al. 1989; van Riper et al. 2002; Vargus 1987; Smits et al. 2005). The presence of exotic biting insects such as *Culex* spp. was identified as a major reason for an increased problem with avian pox infections in Hawaii, the Galapagos and the Canary Islands.

While the overall prevalence of avian pox was found to be low in silvereyes in this study (< 2% in 2010 and 3.7% in 2011), I observed that the disease was more prevalent during the early winter of 2011 in a sample population of silvereyes located in Moana. In temperate zones, epizootics of avian pox are more common in summer and early autumn (van Riper & Forrester 2007). Given the temperate, seasonal nature of the climate in the South Island of New Zealand an expected outcome was for a higher prevalence of avian pox during the summer and autumn months. My survey of silvereyes in Christchurch during the months of summer 2011 did not show any increase in prevalence of pox and similar results were obtained for silvereyes included in the across species survey carried out in Kaikoura in the spring and summer of 2012. A number of variables play a part in the epizootiology of avian pox including; host susceptibility,

vector density and climate (van Riper & Forrester 2007). My observations of an elevated level of avian pox in a population of silvereyes found in Moana, a high rainfall area of the west coast where biting insects are present in high numbers (pers. obs.) support the suggestion that vector density has an influence on the rate of avian pox infection and indeed may be a major contributing factor for infection rates in New Zealand.

The contention that bird populations that have passed through a genetic bottleneck are more susceptible to disease is discussed in chapters 2 and 3. In New Zealand this issue is worthy of discussion because so many bird populations throughout the country have either been dramatically reduced in size or were initially established in small founder groups. While European passerine birds were successfully introduced to New Zealand in the mid nineteenth century and still thrive today (e.g. thrushes and finches), many were shipped out in small numbers (total releases were between 29 and 653) (Veltman et al. 1996). Rapid declines in native and endemic bird species since European settlement from the early nineteenth century mean that population bottlenecks have affected a large proportion of New Zealand's avifauna. Some species that have experienced severe bottlenecks have already received attention from researchers where vulnerability to disease was connected to this phenomenon. Avian pox is one of the diseases identified as having a negative impact on bottle necked species, for example, the South Island saddleback (*Philesturnus carunculatus carunculatus*) and the Chatham

Island black robin (*Petroica traversi*) (Hale 2008; Tisdell & Merten 1988). In my investigation into the prevalence of avian pox I did not detect any increase in the prevalence of avian pox in introduced species, all of which have experienced population bottlenecks. Silvereyes have also passed through a population bottleneck as small flocks have only recently colonised New Zealand, however, I found that apart from the increased prevalence of avian pox in silvereyes in the west coast region, there were no differences in prevalence when comparisons were made with other reports of avian pox in white-eye species overseas.

In continental areas avian pox is considered to be a disease with a long history of co-evolution between virus and host where the prevalence of avian pox in wild bird species has commonly been documented at less than 1.5% (van Riper & Forrester 2007). It is possible that European species introduced to New Zealand continue to maintain resistance to the strains of avian pox circulating in New Zealand because they are already familiar with them. In my investigation native or endemic birds were caught in relatively low numbers and I was unable to survey endangered endemic species. Whether introduced strains are more pathogenic in the case of New Zealand's endemic species requires further investigation. Very recently Ha et al. (2012) found examples of avian pox in 15 different bird species in New Zealand, 10 of which were endemic and one native. She also determined that many of the isolated strains matched closely to strains identified overseas. With the knowledge that a range of avian pox strains are established in New Zealand and that many species of biting

insects are prevalent in this country including *Culex quinquefasciatus*, a vector for avian pox, this is arguably an important area of research as conservation managers may need to develop protocols to mitigate against potential impacts from avian pox.

To more accurately gauge the prevalence and impact of avian pox on New Zealand bird species knowing that the epizootiology of the disease is influenced by a range of biotic, and abiotic factors, requires long term investigation and this was not possible within the scope of my study. My review of the literature on the prevalence of pox in passerines confirmed that there is a pattern of variability in prevalence of the disease between seasons and years. An ongoing national survey to measure the number of common birds observed in New Zealand's parks and gardens (chapter 3), highlighted changes in silvereye numbers from one year to the next. While there could be any variety of reasons for these observed fluctuations other than disease, attributing a reported decline in silvereyes in 2009 to an epizootic of avian pox cannot be ruled out. If avian pox is truly a key factor in silvereye population fluctuations then a long-term study taking into account biotic and abiotic factors, covering many locations over a period of several years will better determine whether this disease is indeed a major contributing factor to changes in population. A longer-term investigation is more likely to detect a local or larger scale epizootic and allow connections to be made with triggers for such events, for example, higher rainfall or an increase in temperature. Sample bias may have been a factor in this study because birds

debilitated by pox lesions may be less likely to be caught. In the cases of the more serious diphtheritic form infected individuals are likely to die quickly and unlikely to be caught at all. The restriction of conducting surveys in only one location in limited seasons each year also added to the problem of sampling bias and could be addressed in future investigations.

An attempt to assess the impact of avian pox on infected silvereyes was achieved through observations of differences in body condition, morphology and haematology (chapter 4). A low capture rate of silvereyes presenting with pox lesions meant that providing statistically significant observations for differences in the above attributes was not possible. Although not significant, the biggest difference was seen in haematological attributes where white blood profiles, specifically absolute proportions of heterophils and eosinophils were lower. Low numbers of heterophils (heteropenia) is considered to be indicator of severe stress (Maxwell 1993), a low eosinophil count (eosinopenia) is also an indication of stress (Monks & Forbes 2007). Further sampling of silvereye populations and perhaps collaboration with other researchers is required in order to build a data base large so that statistically significant results can provide a better understanding about the impact of avian pox on the health of silvereyes and other species. While limited by a very small sample of blood smears from silvereyes infected with avian pox it is worth noting some of the dramatic differences seen in the morphology of both erythrocytes and leucocytes in a smear collected

from one of infected individuals (chapter 4). Changes in morphology, such as blebbing and cytoplasmic vacuolisation consistent with the high stress conditions created during a viral infection were observed. In the same smear I observed large numbers of rubricytes (young erythrocytes), and indication of regenerative anemia a condition associated with parasitism (e.g. *plasmodium*) and bacterial infections (Dein 1986; Campbell 1994).

Direct observation of pox lesions found on infected silvereyes revealed that in most cases lesions were located on the legs and toes. In one infected individual parts of digits were missing and necrotized tissue could be clearly seen indicating the onset of a secondary bacterial infection (Chapter 4). In a juvenile silvereye, a lesion located on one leg was so large that the tarsus had become deformed. Several studies of avian pox in a given species have shown that juveniles are more susceptible to avian pox than adults (e.g. Vargus 1987; Buenestado et al. 2004; Senar & Conroy 2004). I did not investigate nestlings or fledgling silvereyes throughout the study and a future investigation that includes juveniles will answer questions about whether young silvereyes are more vulnerable to avian pox as well as providing a better picture about the prevalence and impact of the disease on this species.

It is not uncommon for lesions to appear on the eyes of birds infected with avian pox (e.g. Karstad 1965) and during my investigation I discovered one silvereye with a lesion on the cornea of one eye. Although not measured directly, it has been suggested that eye lesions are most

likely to compromise a bird's ability to forage and fend for itself. Loss of vision, secondary bacterial infections leading to the loss of nails and digits, growth deformities, predisposition to infection from malaria (Atkinson 2005) and comprised ability to successfully find mates (Kleindorfer & Dudaniec 2005) are some of the negative impacts from infection with avian pox. There are likely to be many other fitness costs associated with this disease.

My investigation confirms the presence of pox in silvereye populations in the South Island of New Zealand. As silvereyes are an abundant, widespread species it is important that we have full knowledge about the strains of virus that exist in this species. If silvereyes carry strains that could be devastating to other bird species in New Zealand then their potential to act as a reservoir for avian pox and the part they play in the cycle of this disease certainly warrants further research.

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